

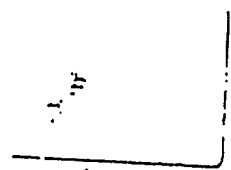
1 agggagaggc agtgaccatg aaggctgtgc tgcctgccc: gttgatggca
 51 ggcttggccc tgcagccagg cactgcccig ctgtgctact cctgcaaagc
 101 ccaggtgagc aacgaggact gctgcaggt ggagaactgc acccagctgg
 151 gggagcagtg ctggaccgcg cgcaaccgcg cagtggcct cctgaccgtc
 201 atcagcaaaag gctgcagctt gaactgcgtg gatgactcac aggactacta
 251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgccca
 301 gcggggccca tgcctgcag ccggctgccg ccacccctgc gctgctccc
 351 gcactcggcc tgcctgctctg gggacccggc cagctatagg ctctgggggg
 401 ccccgctgca gccacacig ggtgtggtgc cccaggcctt tgtgccactc
 451 ctacagaac ctggcccagt gggagcctgt cctggctcct gaggcacatc
 501 ctaacgcaag ttgaccatg tatgtttgca cccctttcc cnaaccctg
 551 acctcccat gggccctttc caggattccn accnaggcaga tcagtittag
 601 tganacanat ccgctgcag atggccctc caaccnttn tgttgnigt
 651 tccatggccc agcattttc acccttaacc ctgtgttcag gcactttc
 701 cccaggaag cctccctgc ccacccatt tatgaattga gccaggttg
 751 gtccgtggig tccccgcac ccagcagggg acaggcaatc aggagggccc
 801 agtaaaaggct gagatgaagt ggactgagta gaaatggagg acaaagatg
 851 acgtgagtc ctgggaggt ccagagatgg ggcctggagg cctggagga
 901 gggggccaggc ctacattg tggggtccc gaatggcagc ctgagcagc
 951 cgtaggccct taataaacac ctgtggata agccaaaa aa222222

FIGURE 1A

MRALLALLMAGLALQPGTALLCYSCKAQVS.NEDCLQV
ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS
QDYYVGKKNITCCDIDLNASGAHALQPAAAILALLPAL
GLLLWGPGL

1994-03-01

FIGURE 1B



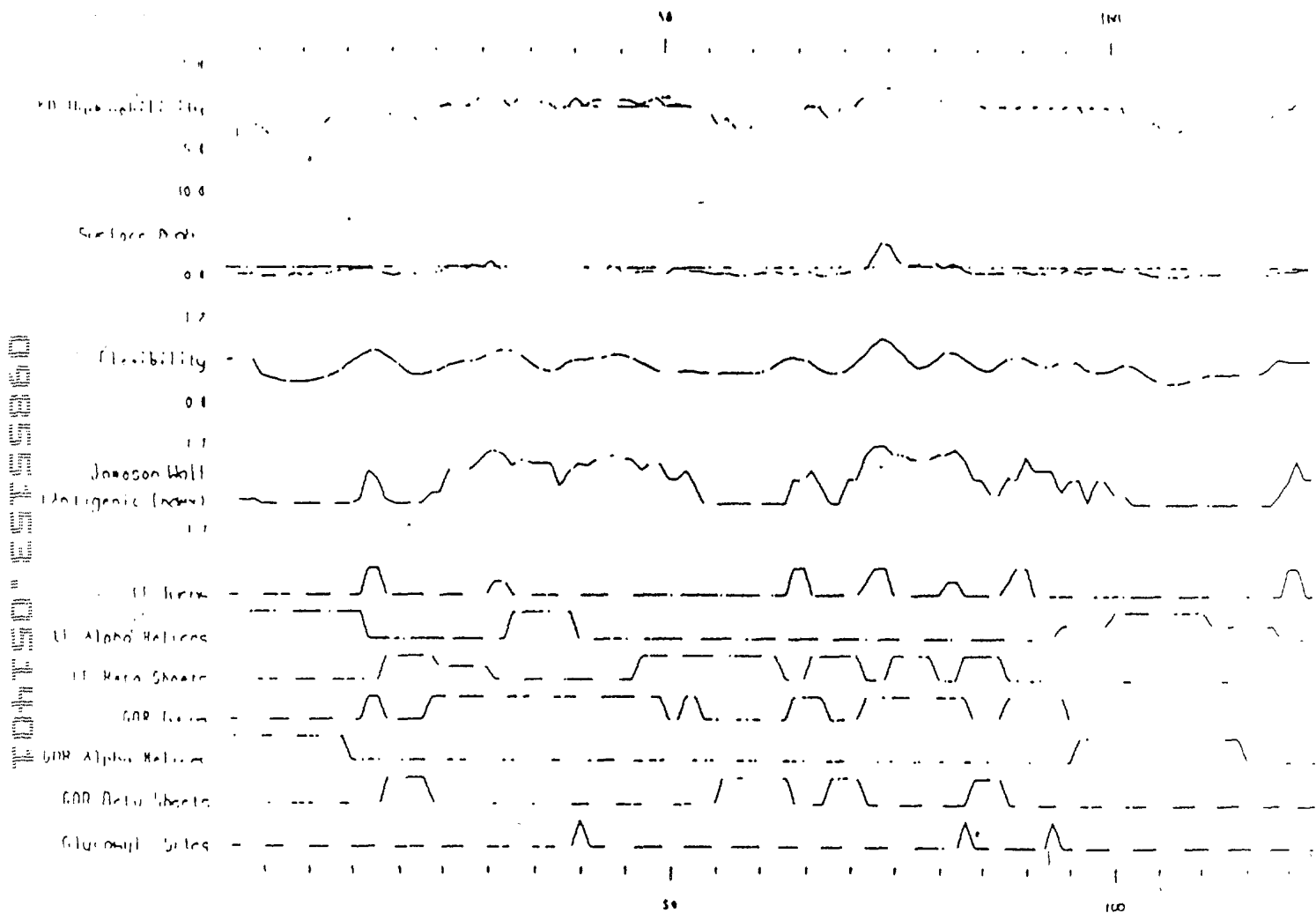


FIGURE 4

↑
signal
sequence

○ = glycosylation
site

✓ GPI signal

FIGURE 5

Western PSCA
 Support to be 80% of
 Normal tissue
 1hr exp

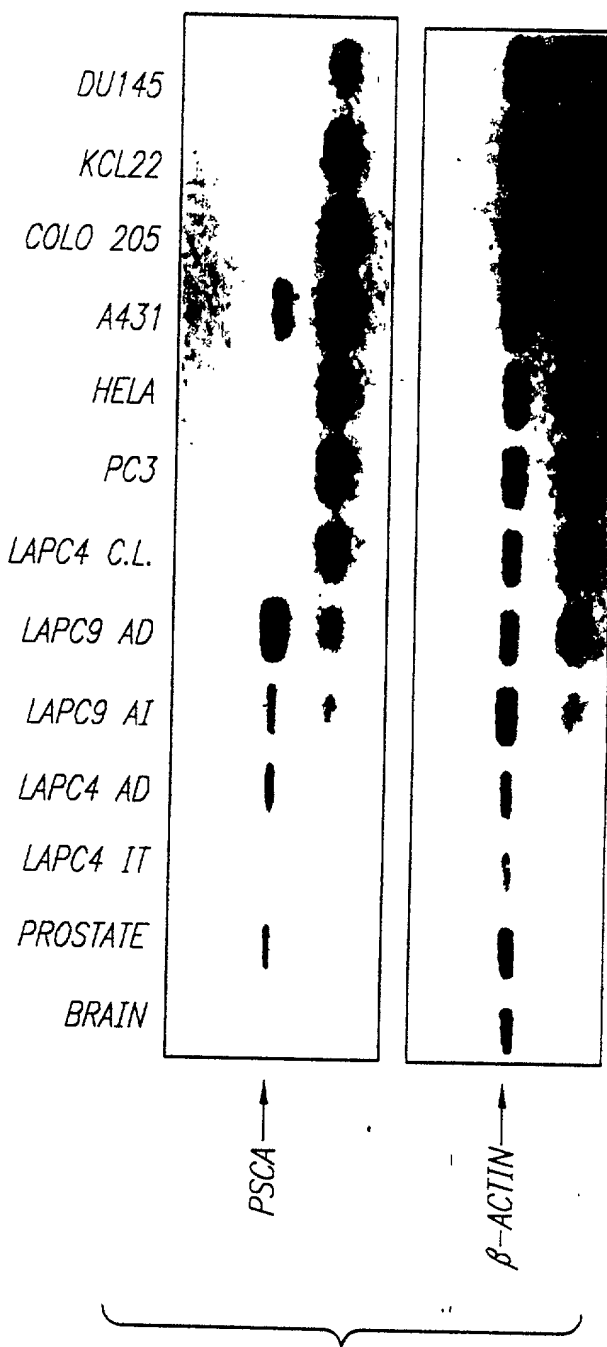
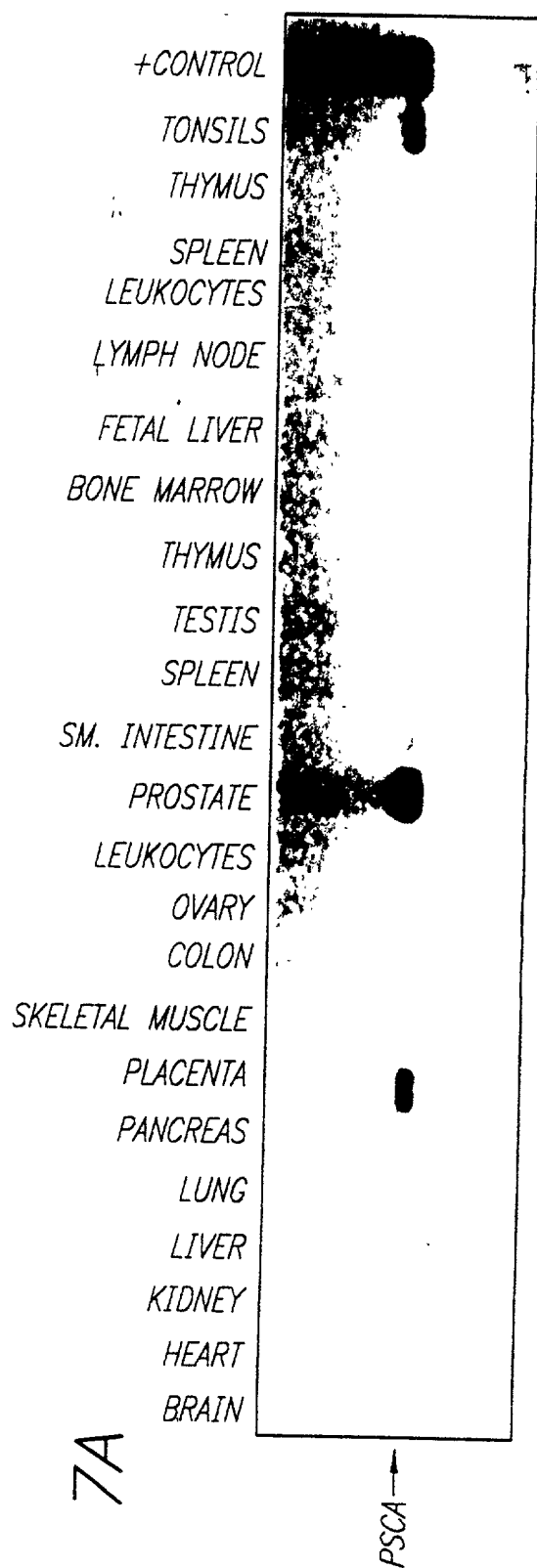
198
 1:100



prostate (Kinner)
 prostate (Baker)
 prostate (Gek)
 Bladder (Kinner)
 Bladder (Gek)
 Bladder (Kob)
 Kidney (NABO)
 Kidney (WU2)
 Testis
 Sm. Intest.

LA PC9

09855153.051401

FIGURE 6



Legend:  untranslated region of PSCA
 translated region of PSCA

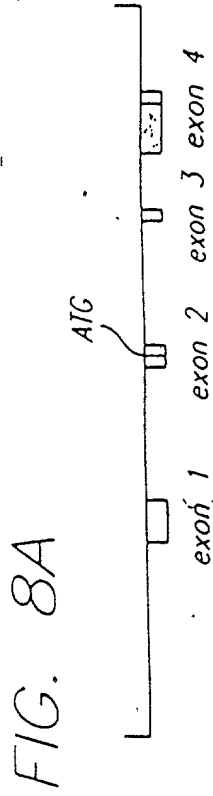


FIG. 8B

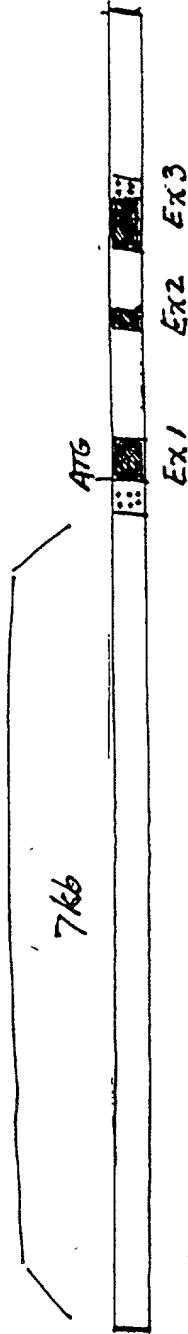
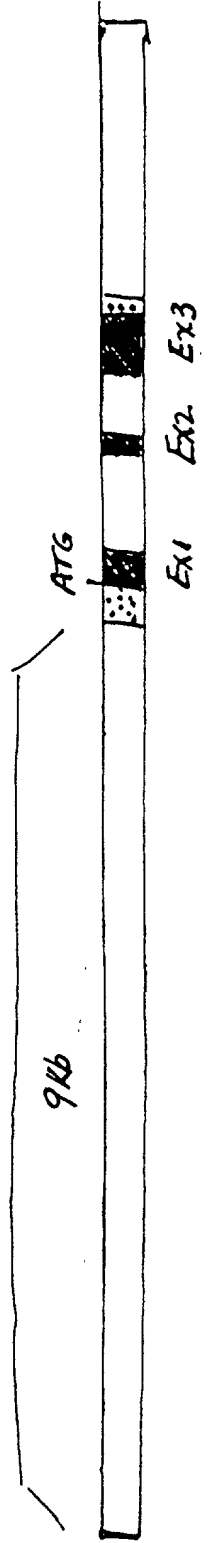


FIG. 8C



human PSCA

FIGURE 8

human PSCA

PSCA / PSA Expression in Benign
Prostate vs. Prostate Cancer Xenograft

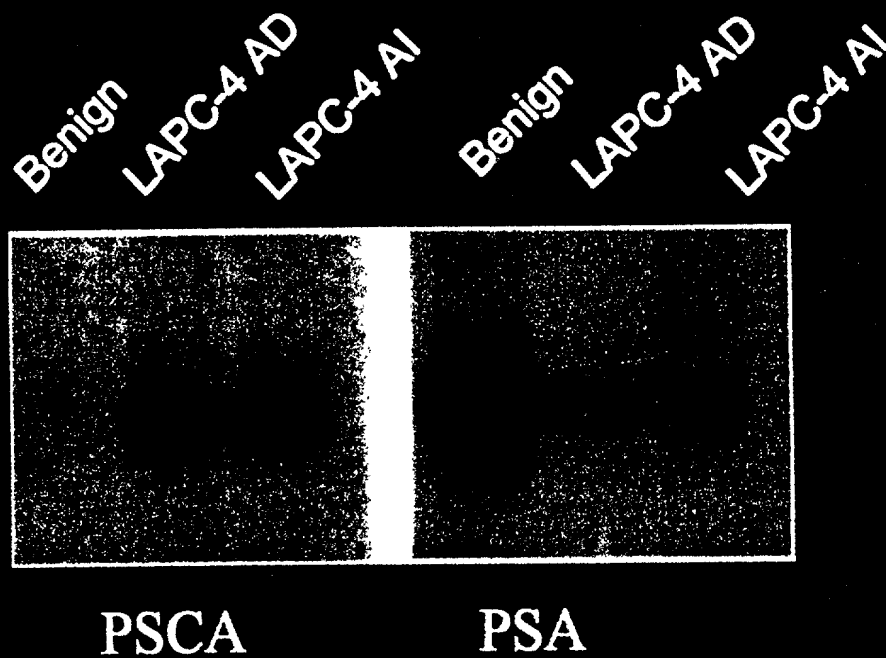
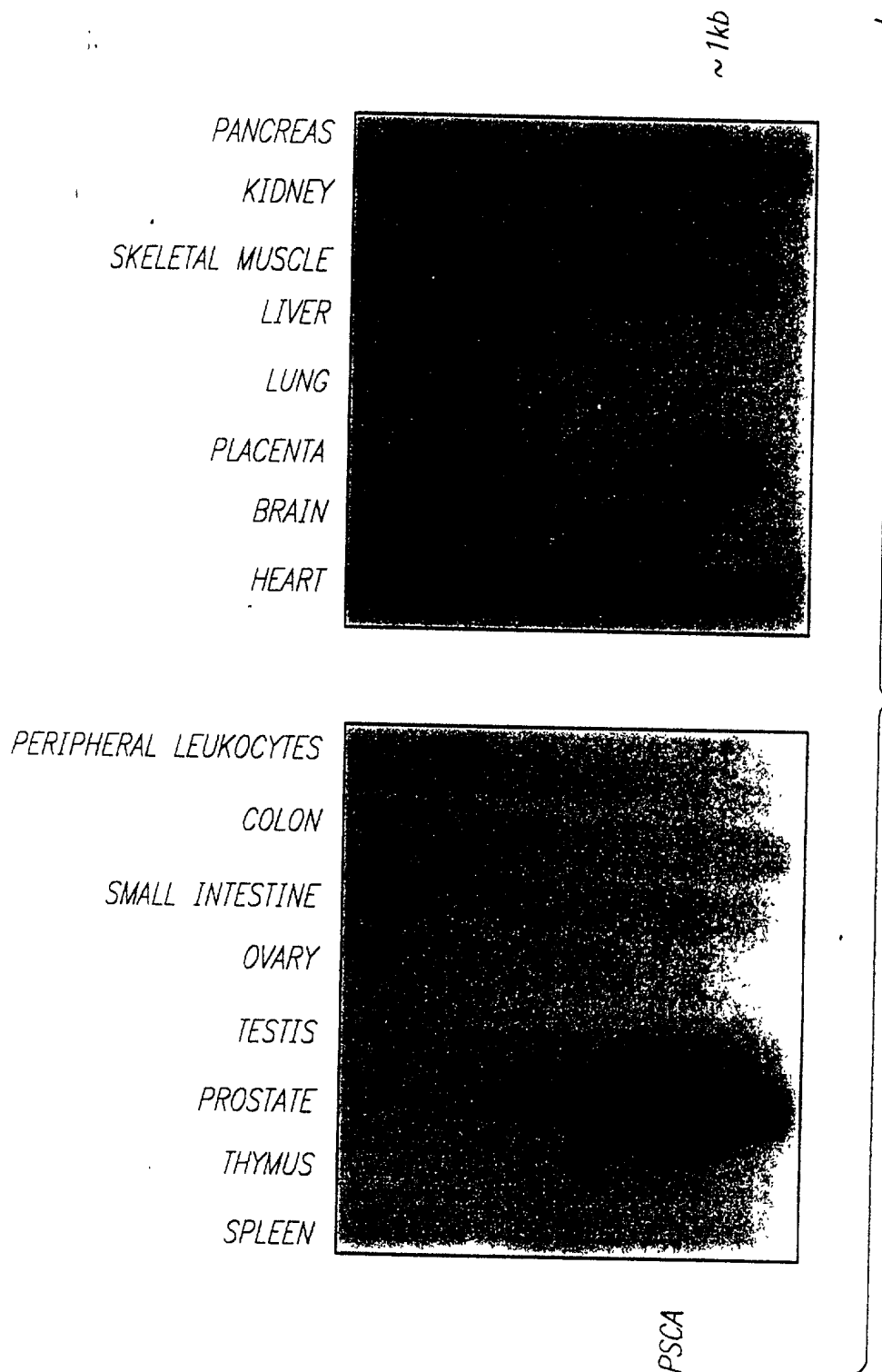
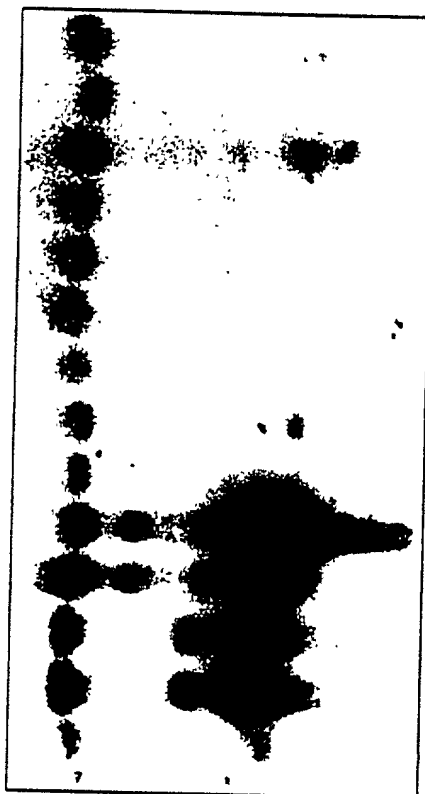


FIGURE 9A



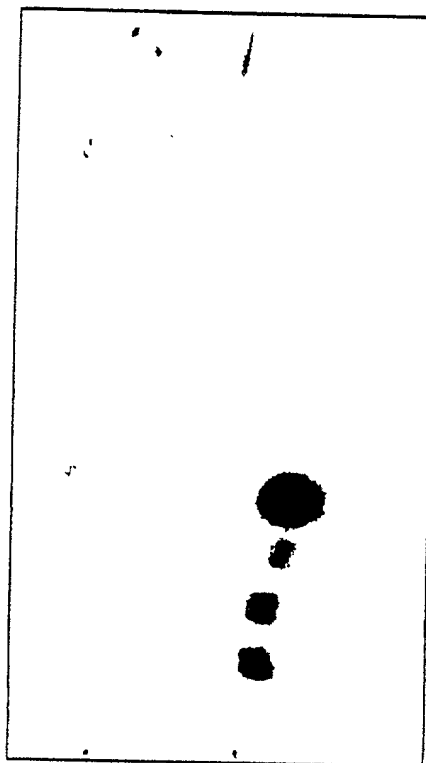
72 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



4 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



PSCA

FIG. 10-1

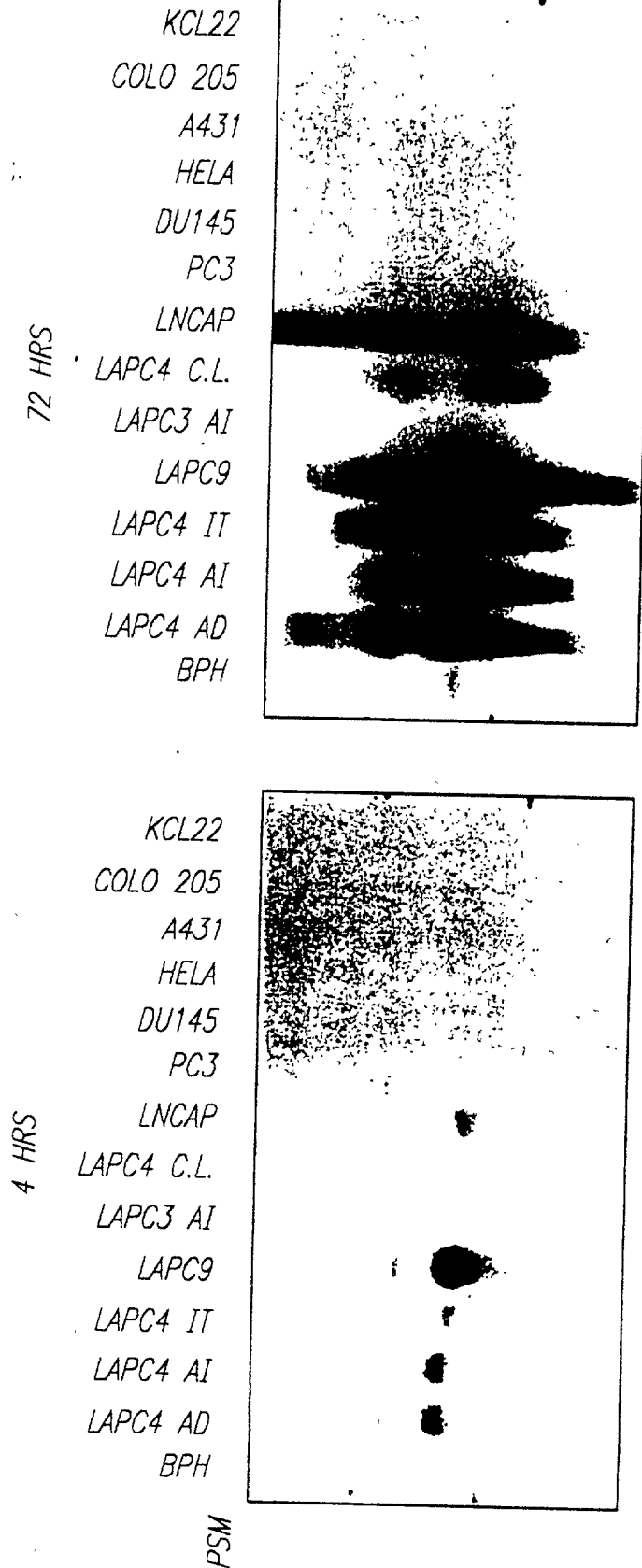


FIG. 10-2

72 HRS

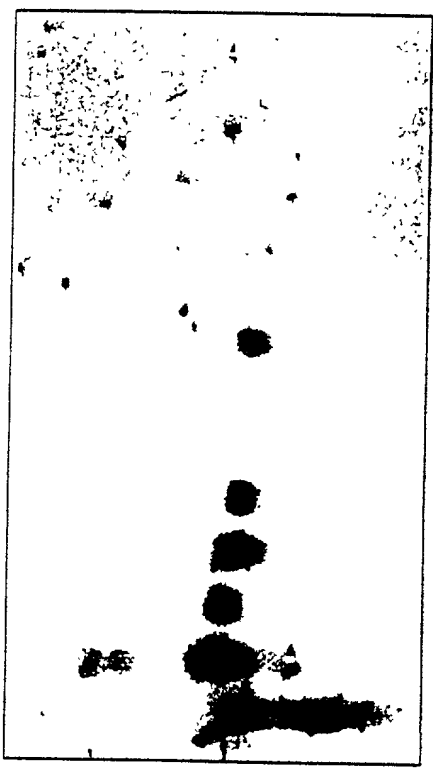
72 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH

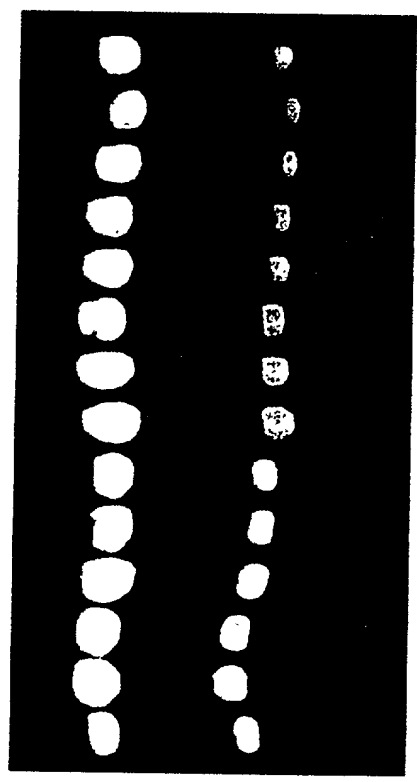


4 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



PSA



EIBR

FIG. 10-3

FIG. 11A



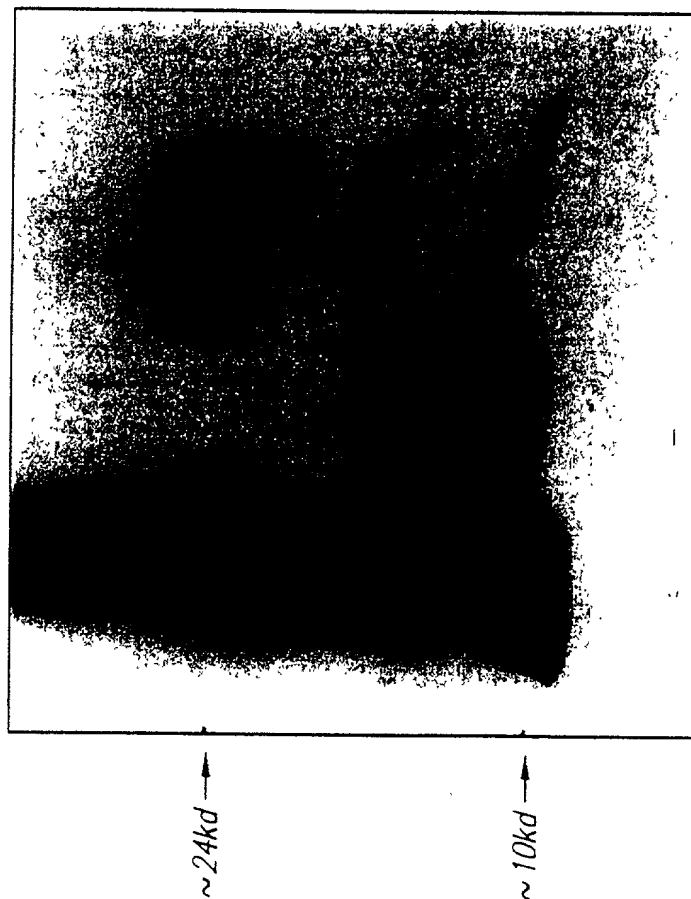
FIG. 11B



FIG. 11C

FIG. 12A

O GLYCOSIDASE
N GLYCOSIDASE F
CONTROL



SECRETED
CELL ASSOCIATED

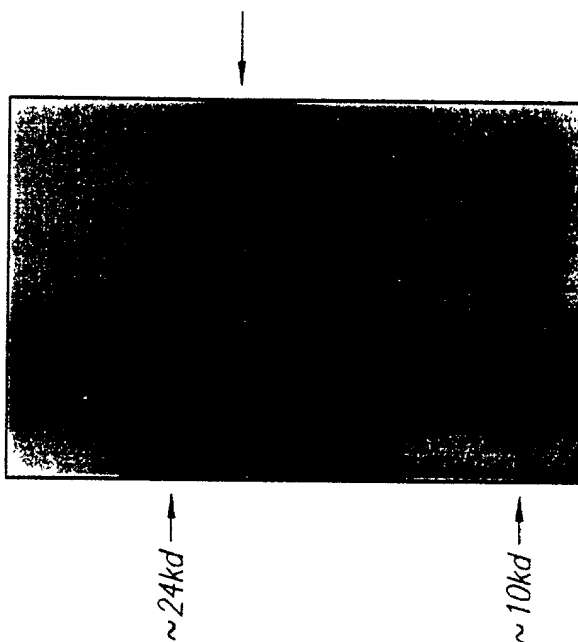


FIG. 12B

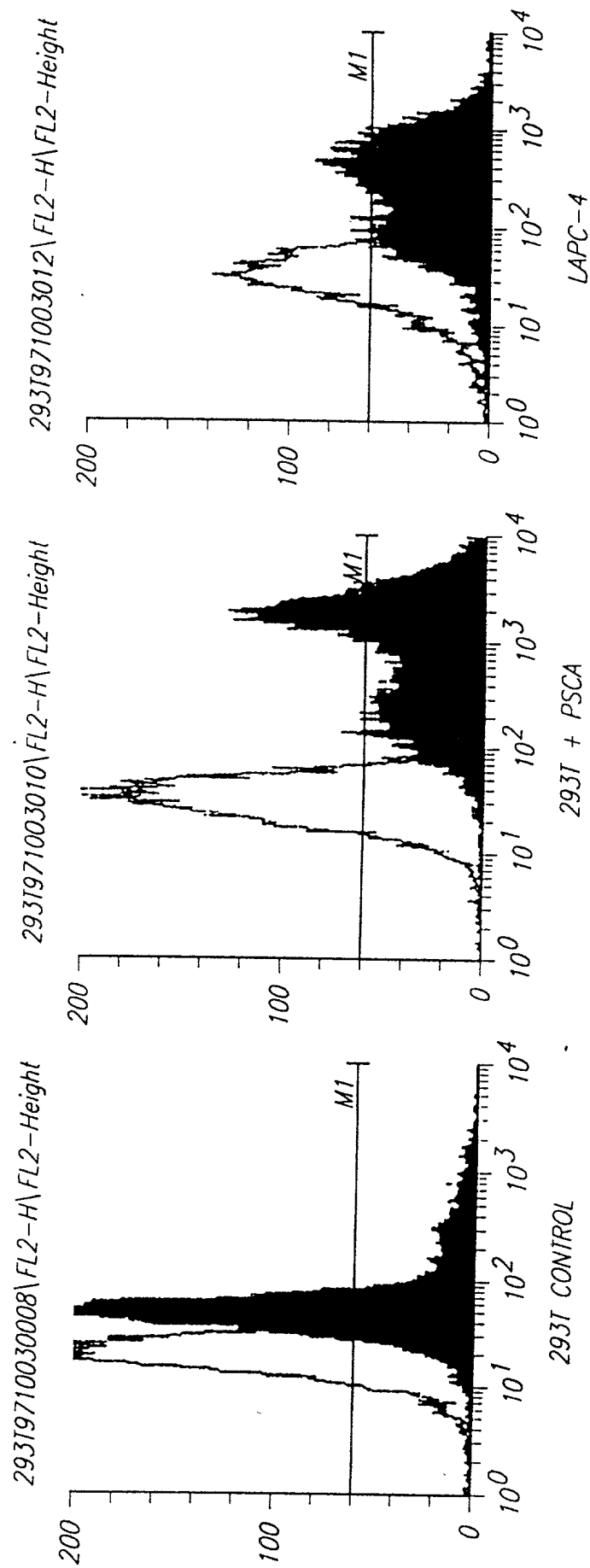
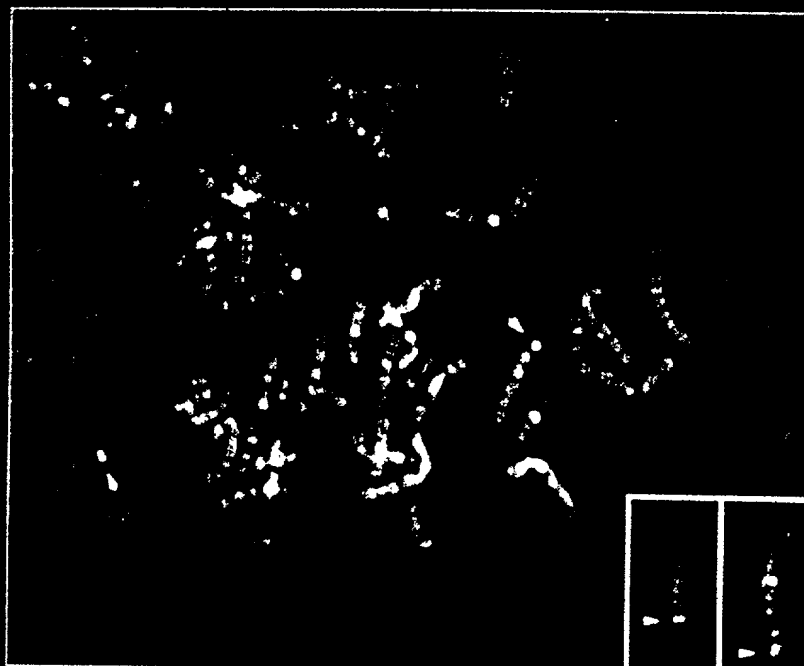


FIGURE 12C

PSCA Maps to Chromosome 8q24.2



Fluorescent
in Situ Hybridization
Analysis of PSCA

FIGURE 13

TOP SECRET

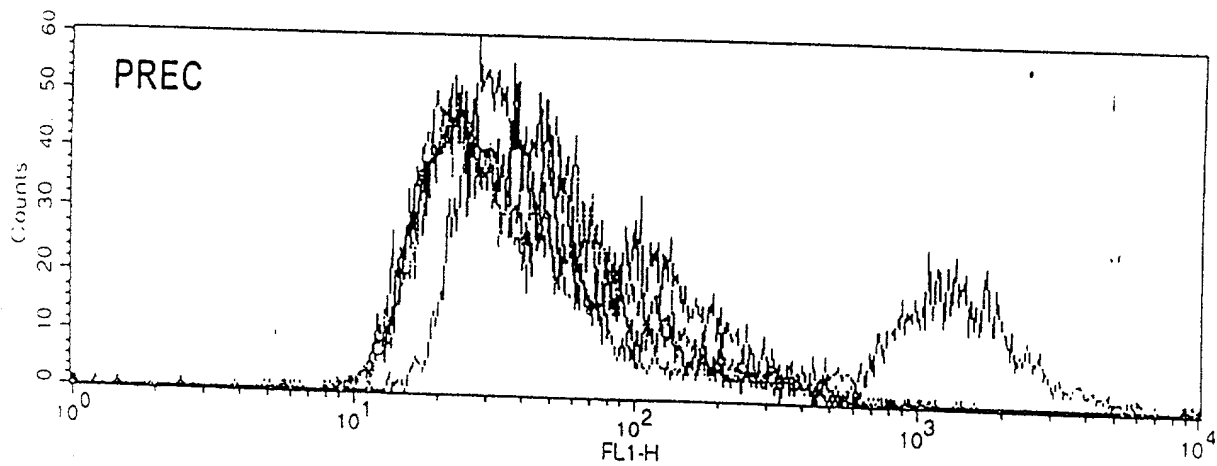
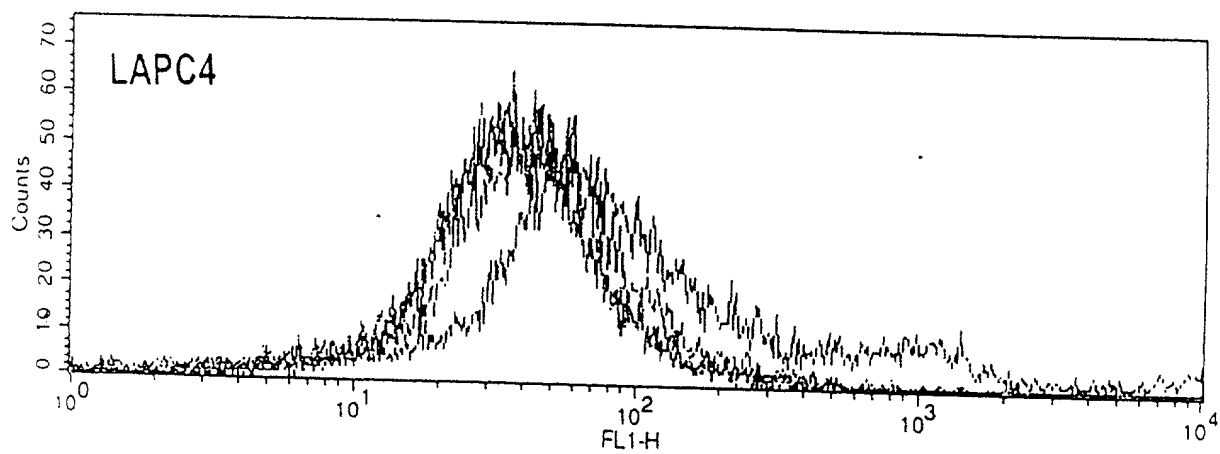
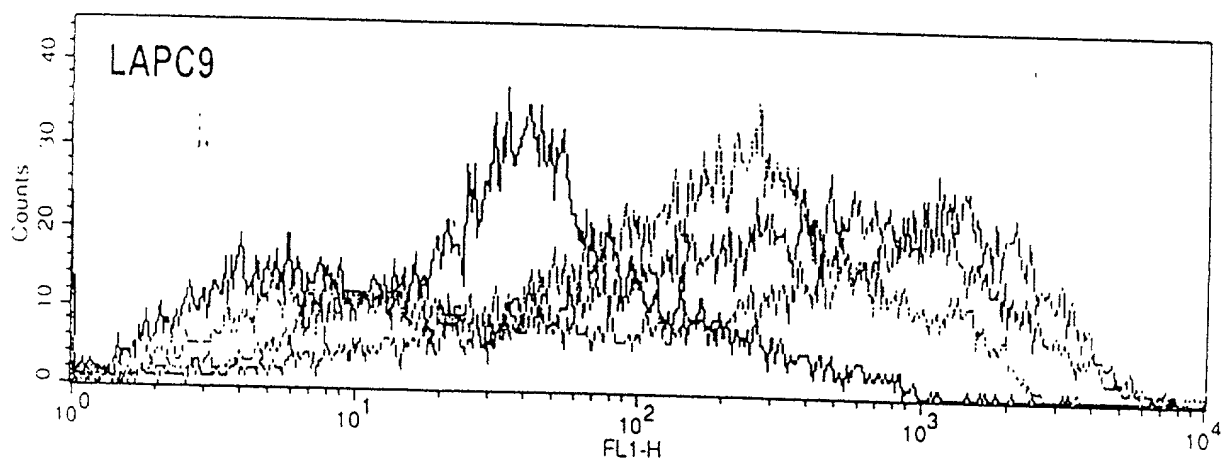


FIGURE 14

A

Epitope map

mAb	Isotype	FL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	2.039	0.007	0.628	0.000
2H9	IgG1 k	1.318	0.863	0.032	0.021
3C5	IgG2a k	2.893	1.965	0.016	0.005
3E6	IgG3 k	0.328	0.024	0.069	0.370
4A10	IgG2a k	2.039	1.315	0.000	0.014
2A2	IgG2a k	1.366	0.733	0.010	0.003
3G3	IgG2a k	2.805	1.731	0.004	0.000

B

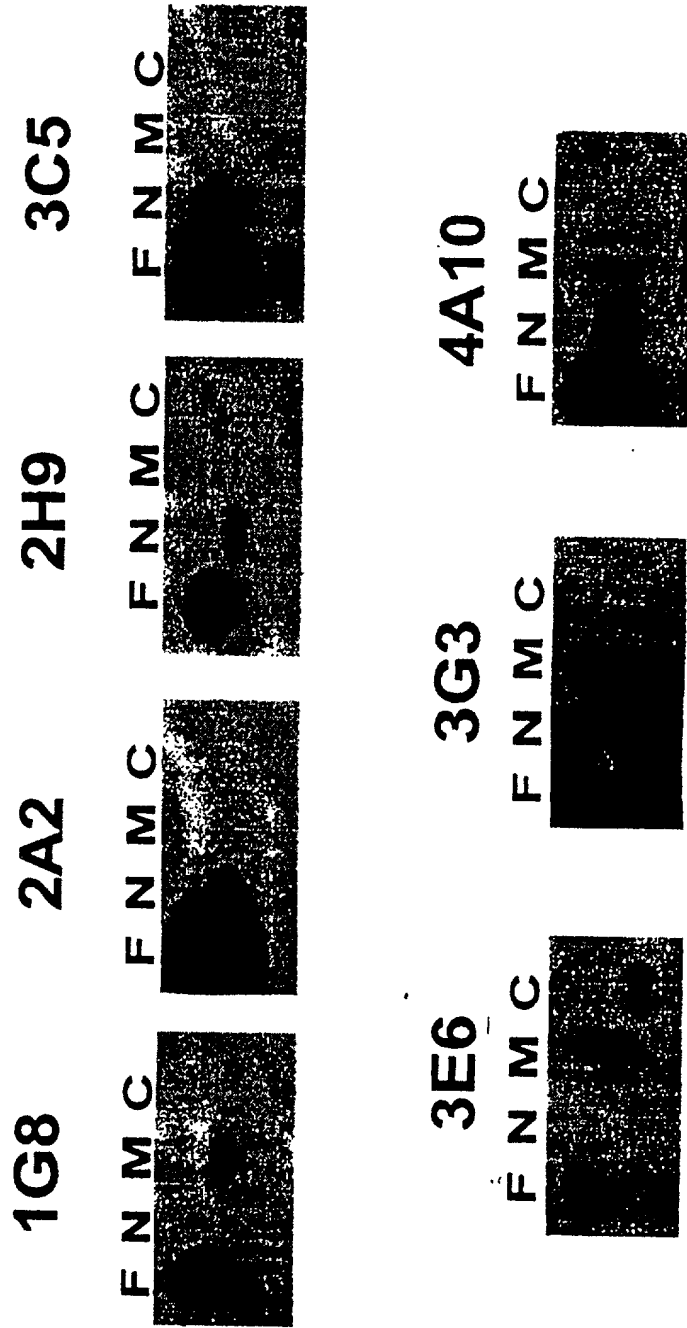


FIGURE 15

[illegible]

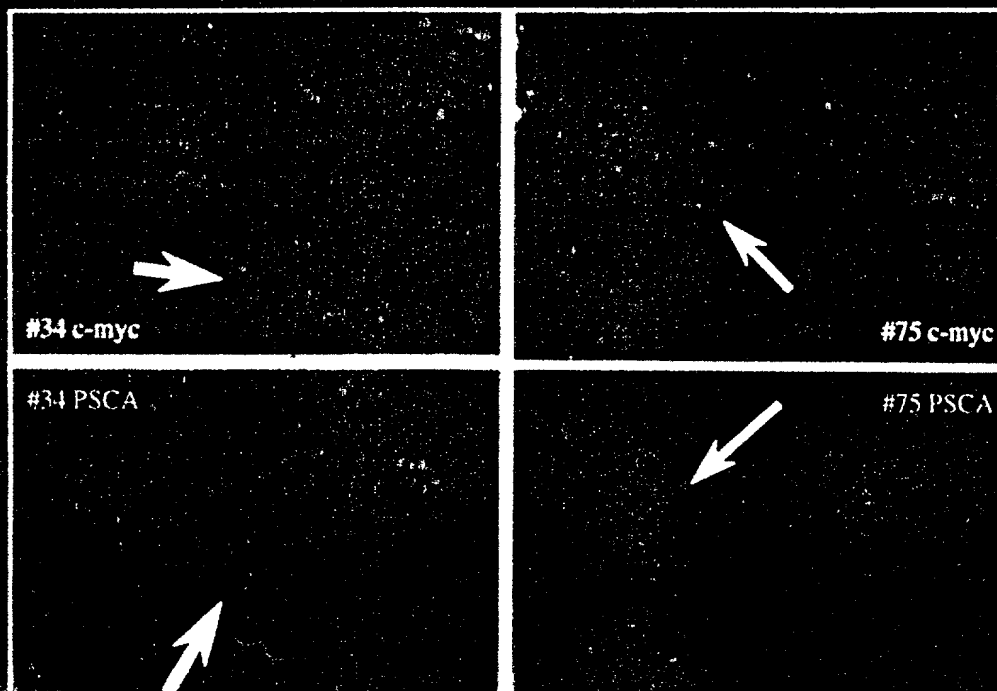
(Reiter, R.E., et al., 1997. *PNAS*)



FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



R. Jenkins

FIGURE 17

0985459 054401

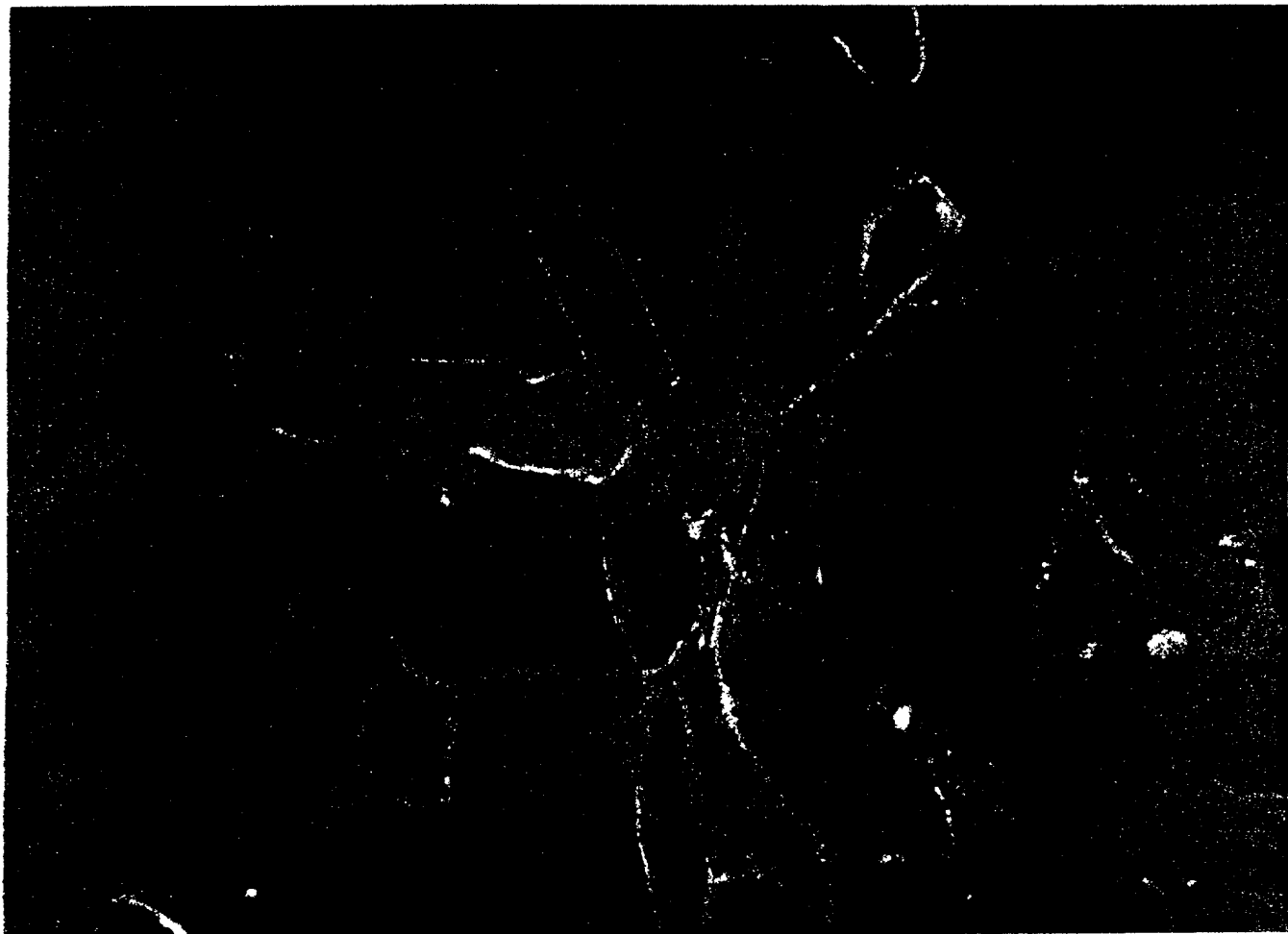


FIGURE 18

TOP SECRET

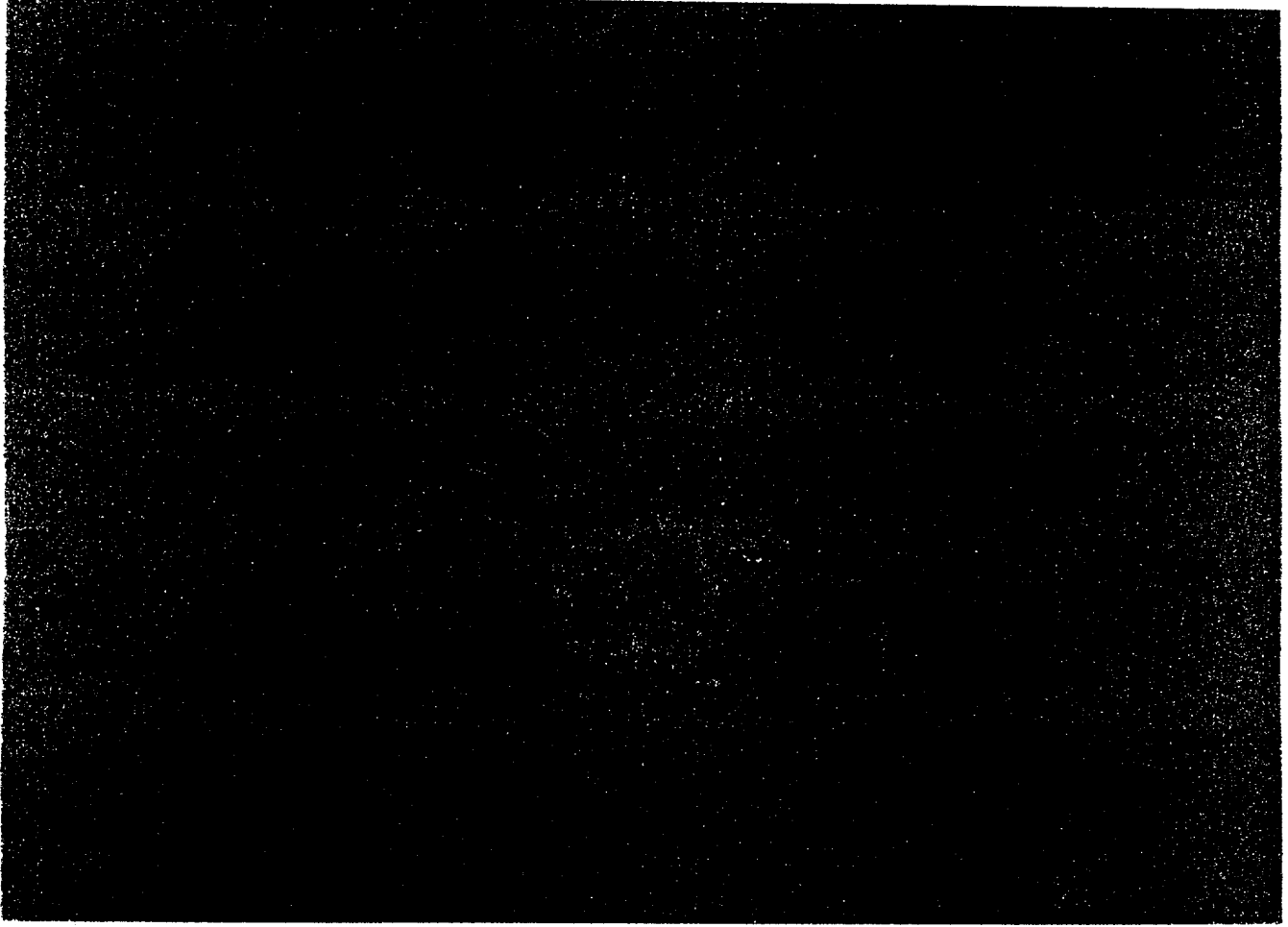


FIGURE 19

10450-05440

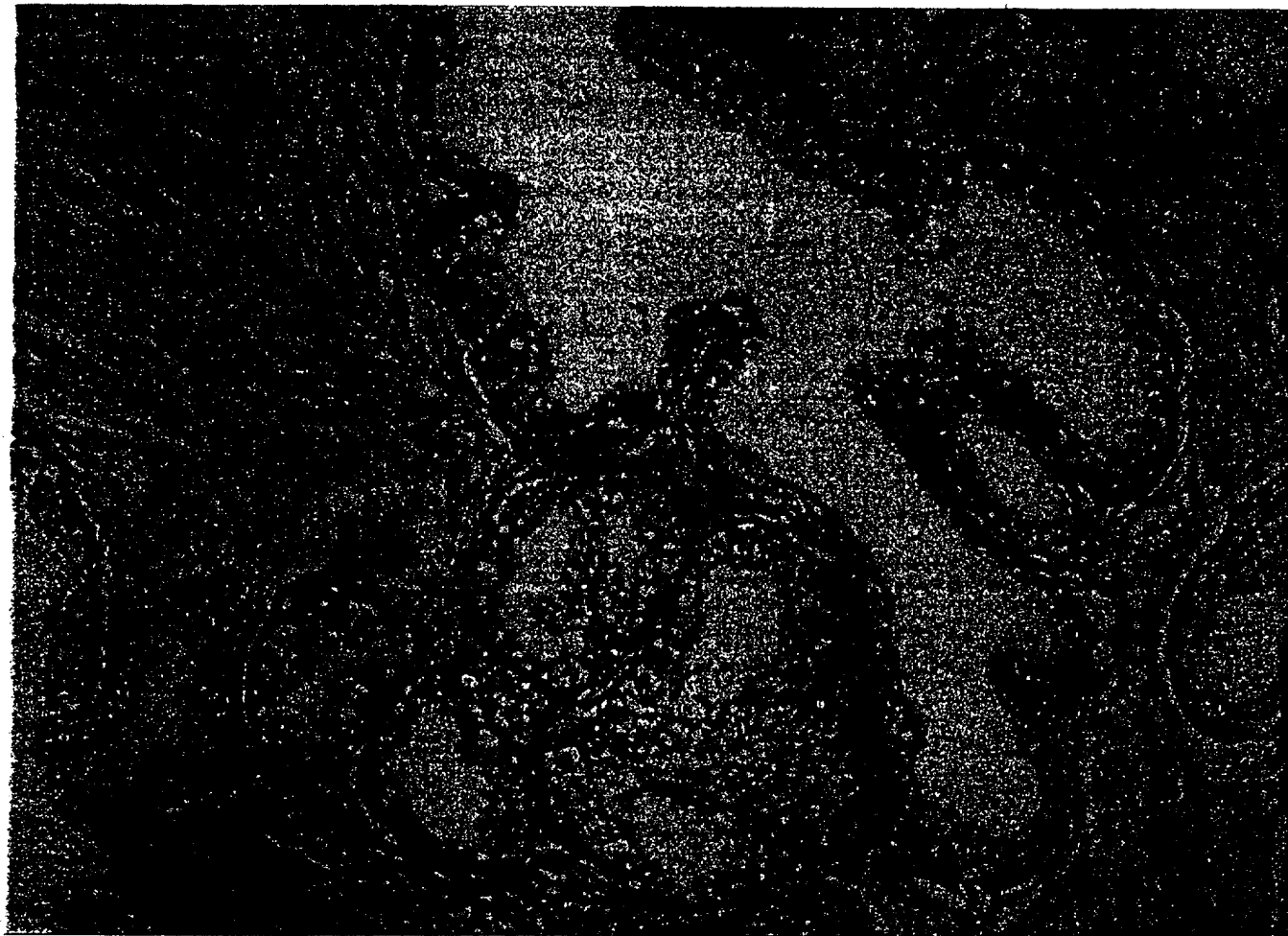


FIGURE 20

1085153-051401
FO-T50-EST5860

PSCA Immunostaining of Primary Tumors

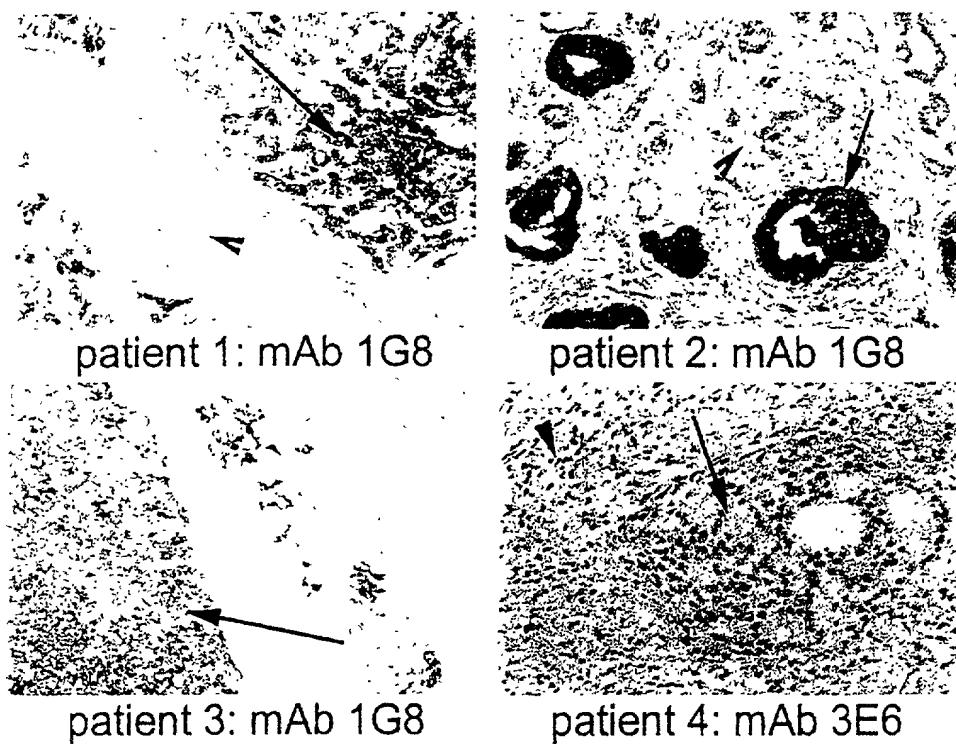


FIGURE 21

FOOTNOTES



FIGURE 22

FOOTNOTES EST 5860



FIGURE 23

0955153 054404



FIGURE 24

0955453-051401

From PSCA
to [unclear] [unclear]

FIGURE 25

T 04750" C 0755350

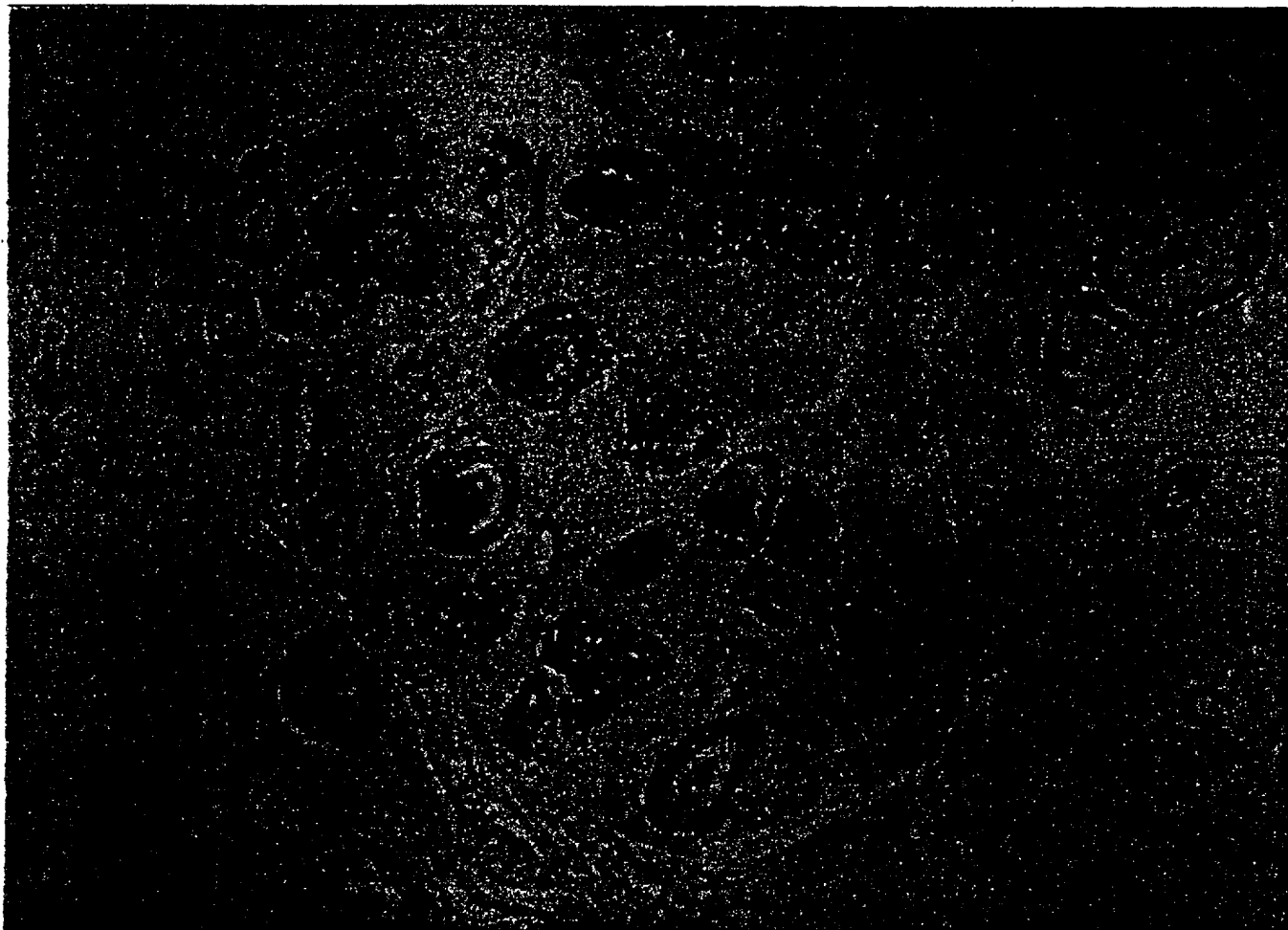


FIGURE 26

10955453-054404

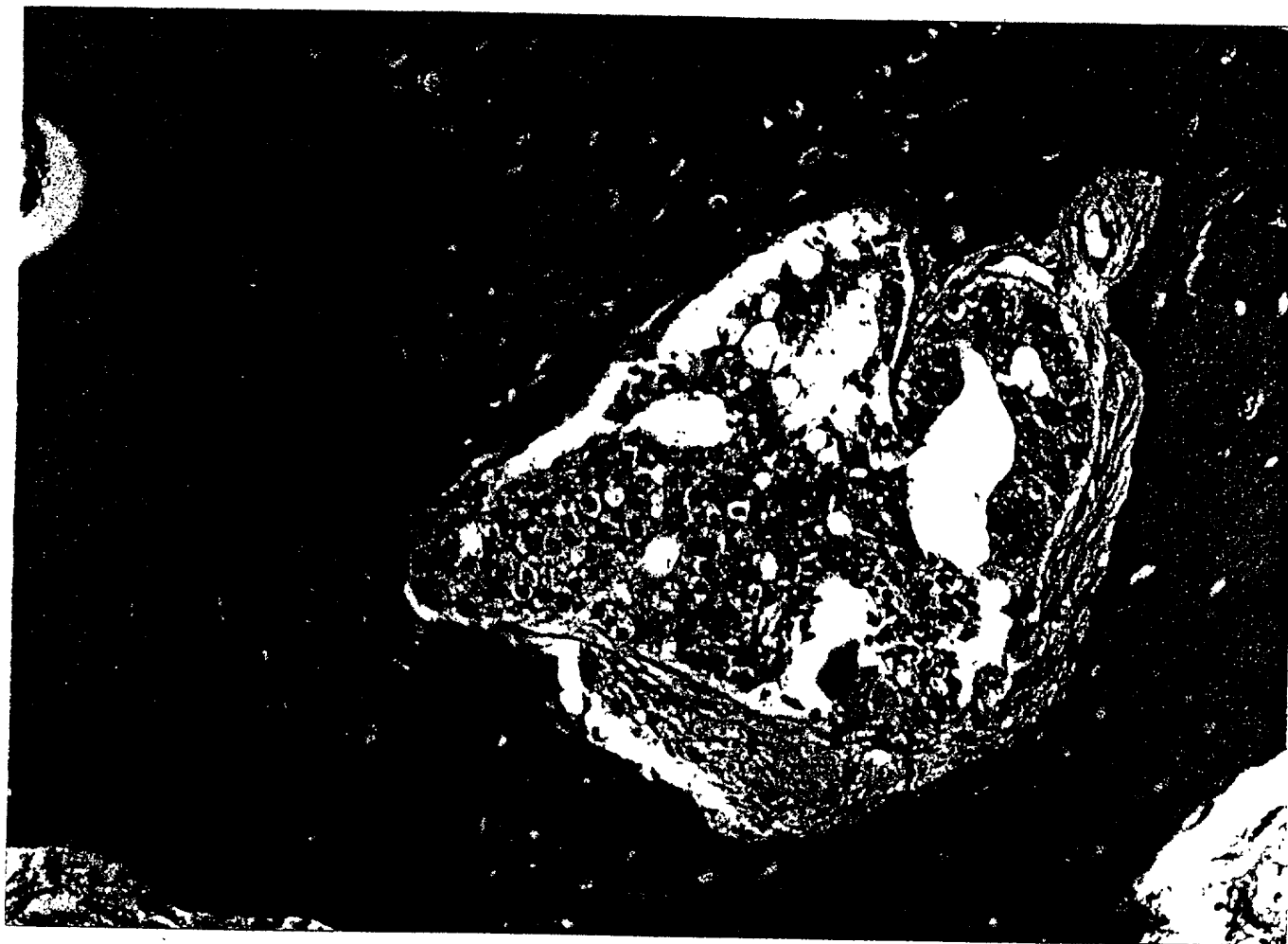


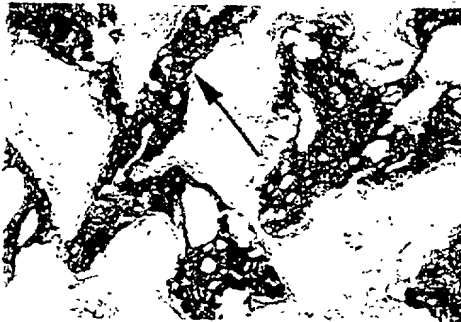
FIGURE 27

TOP SECRET

PSCA Immunostaining of Bony Metastases



Patient 5: H and E
and mAb 1G8



Patient 4: H and E
and mAb 3E6

FIGURE 28

TOP SECRET ESTS 5860

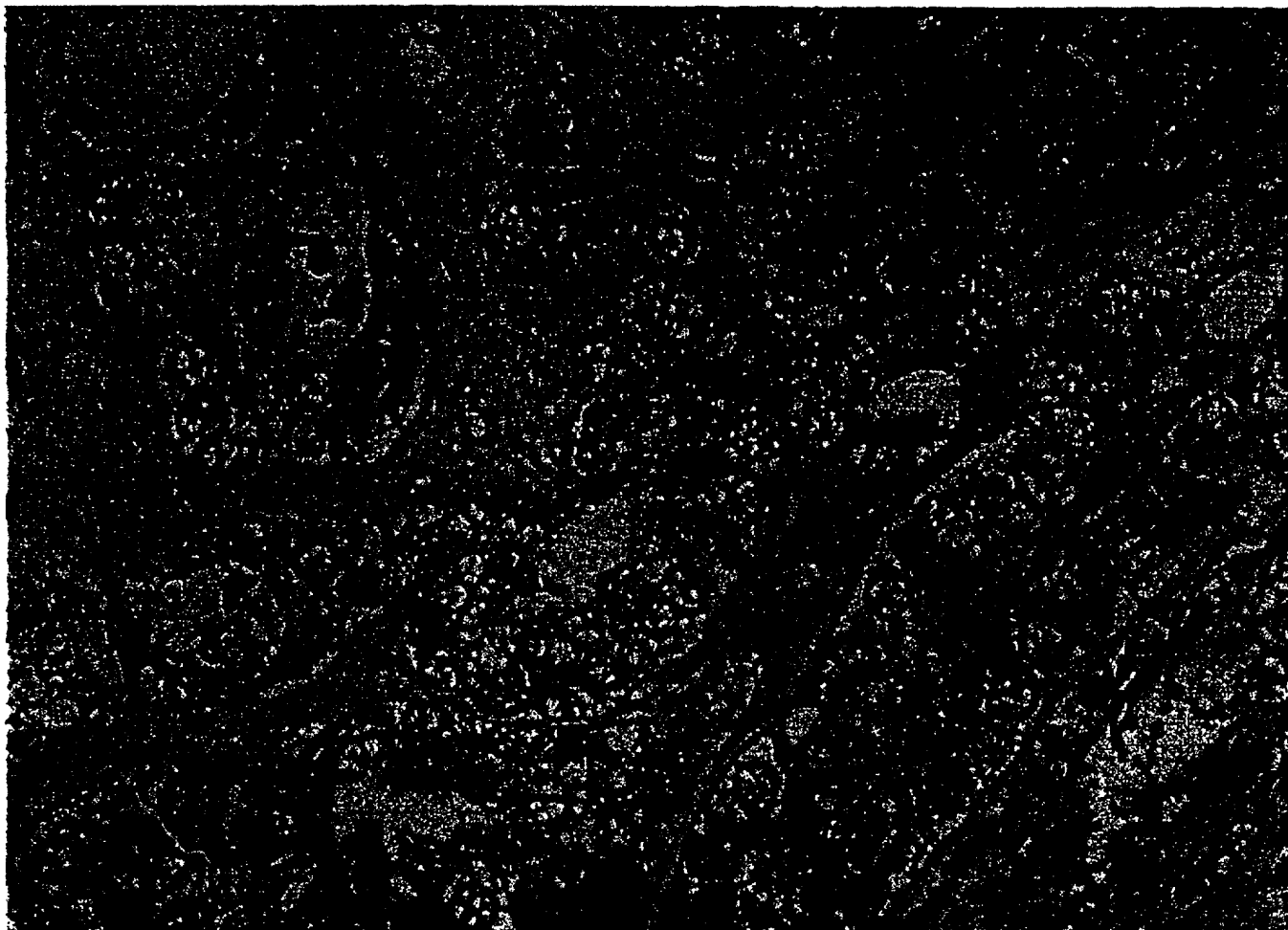


FIGURE 29

0985453-054404

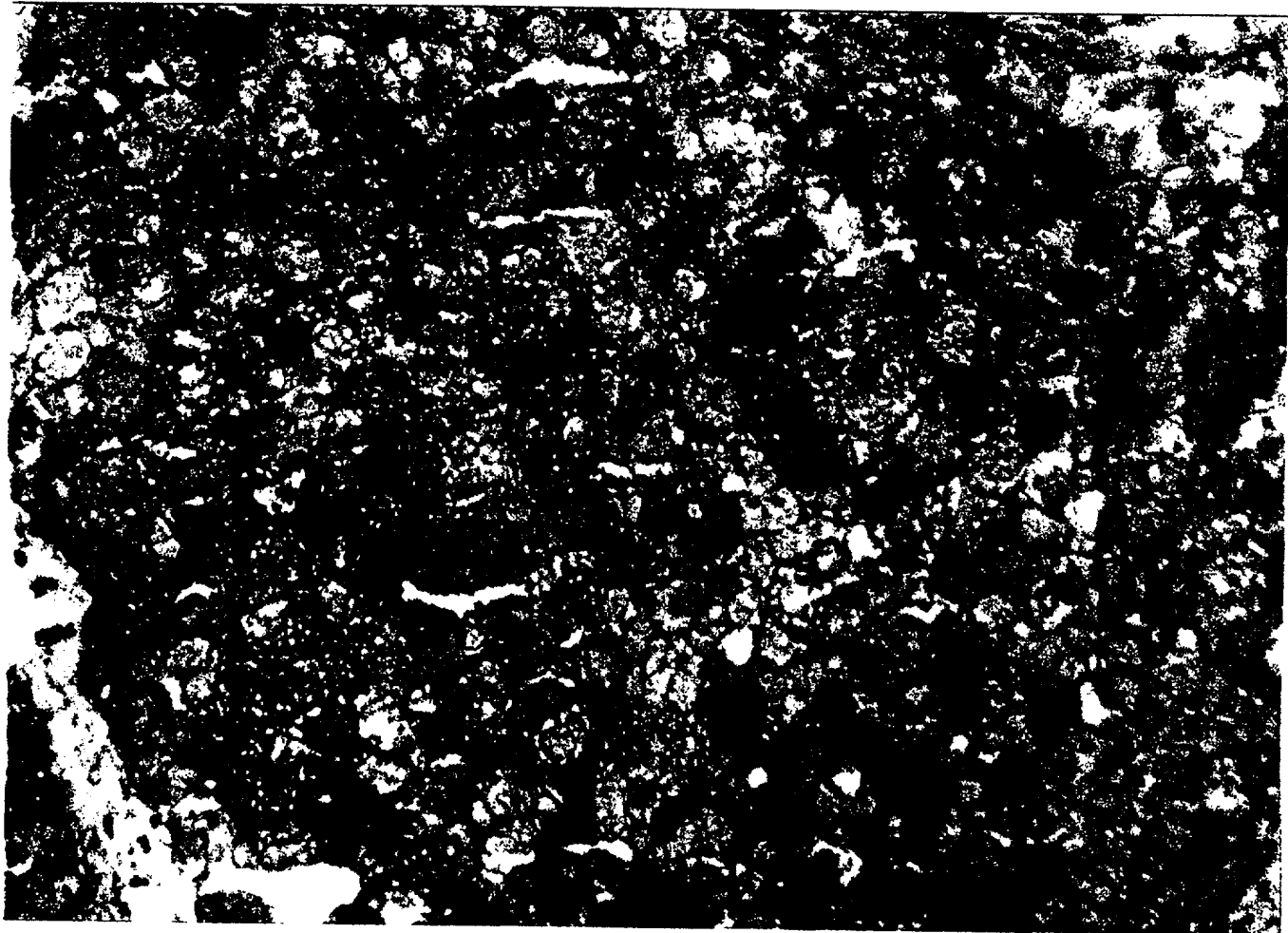


FIGURE 30

09855153 051401

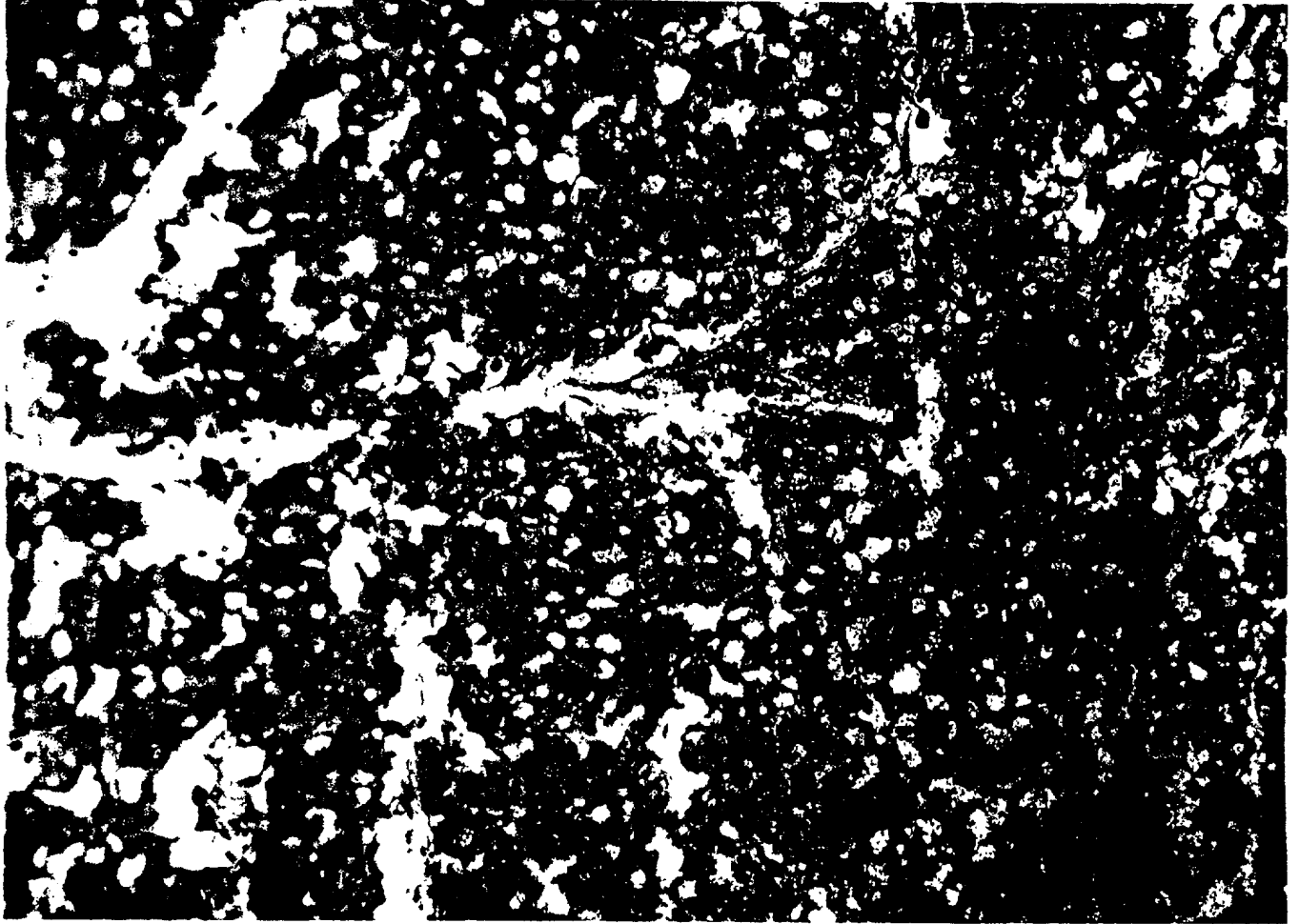


FIGURE 31

0985153-051401
FOUO" ESTS860

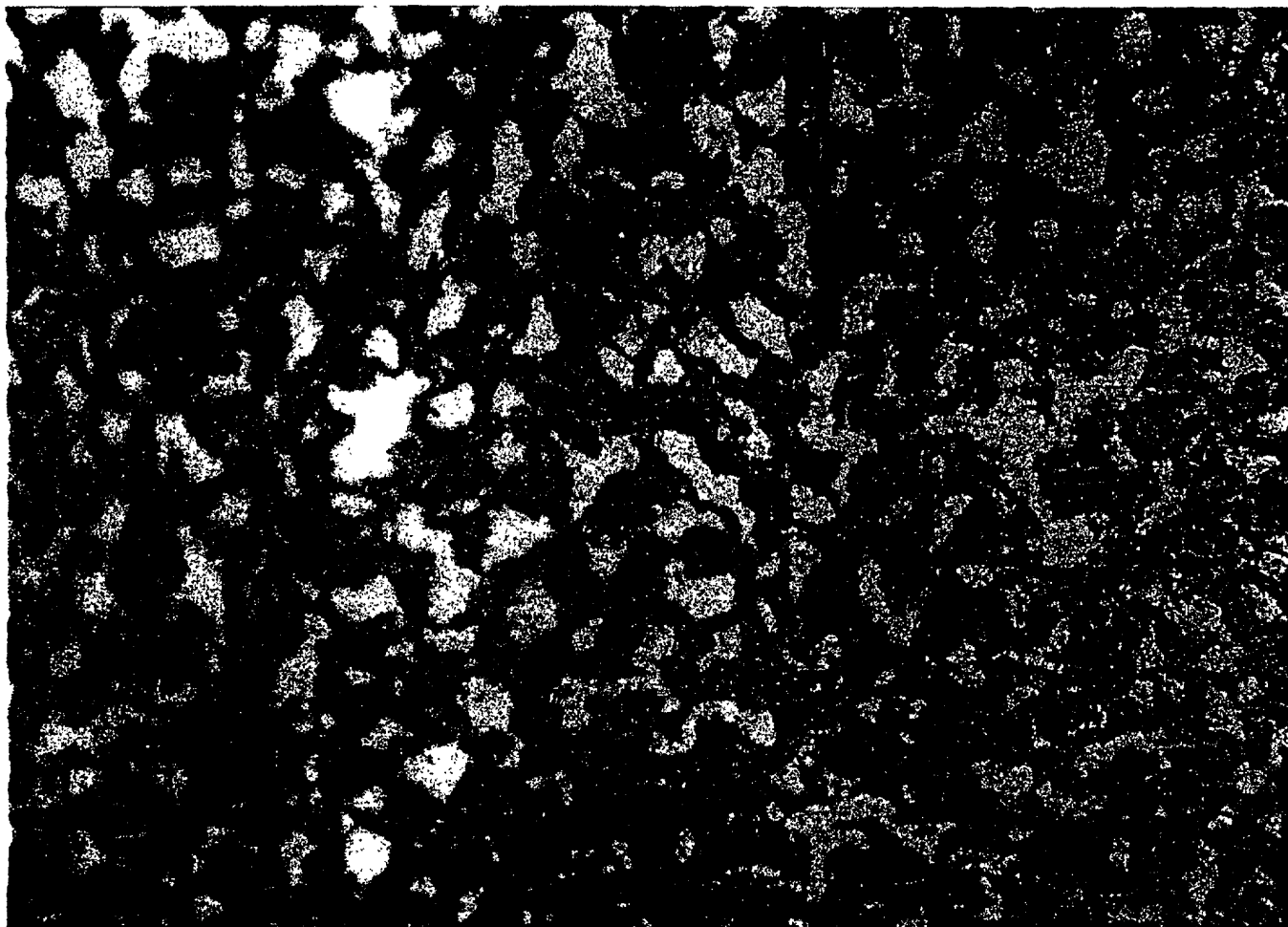


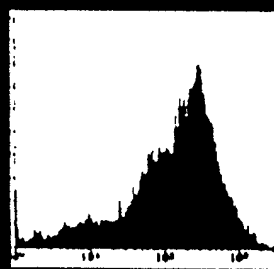
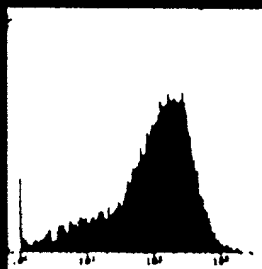
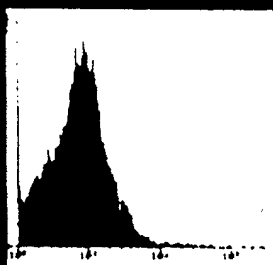
FIGURE 32

PSCA Expression in LAPC-9 Xenograft by FACS

Secondary Antibody

1G8

2H9



4A10

3C5

3E6

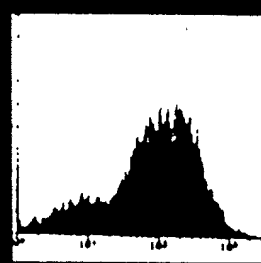
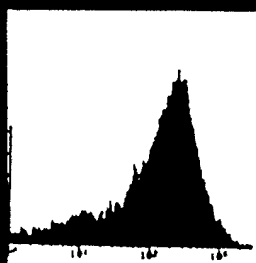
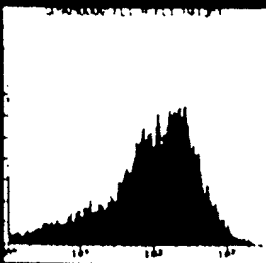


FIGURE 33

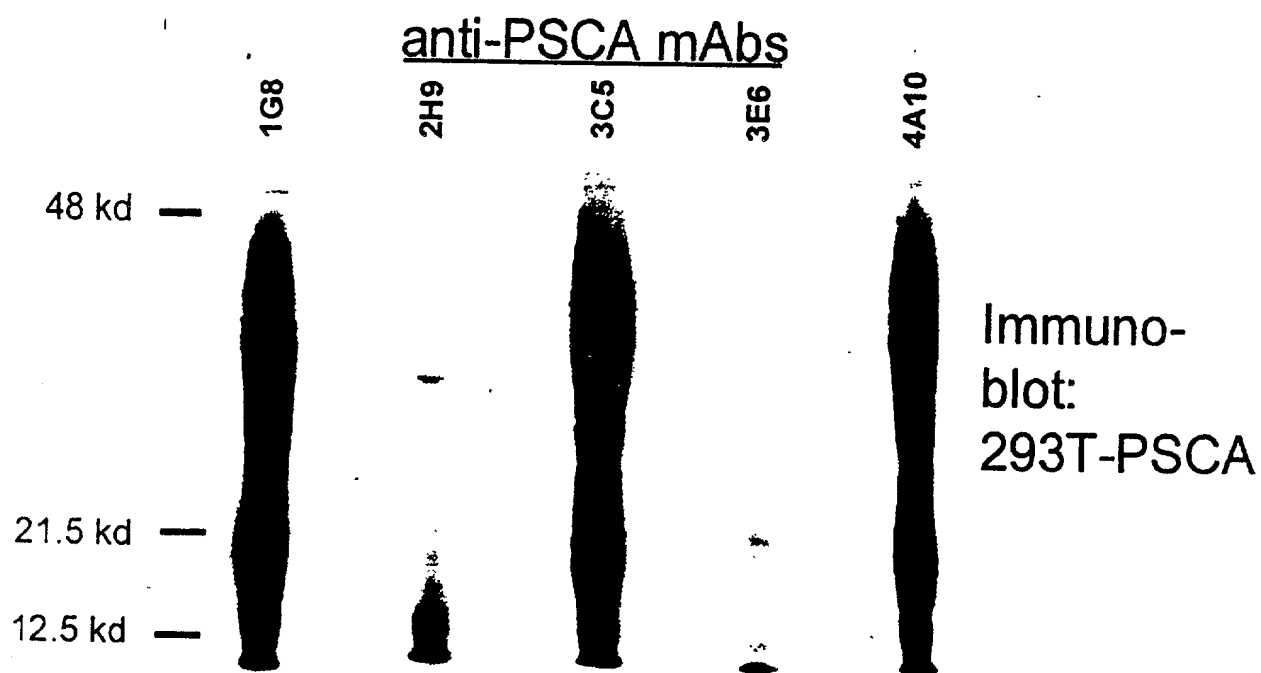


FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

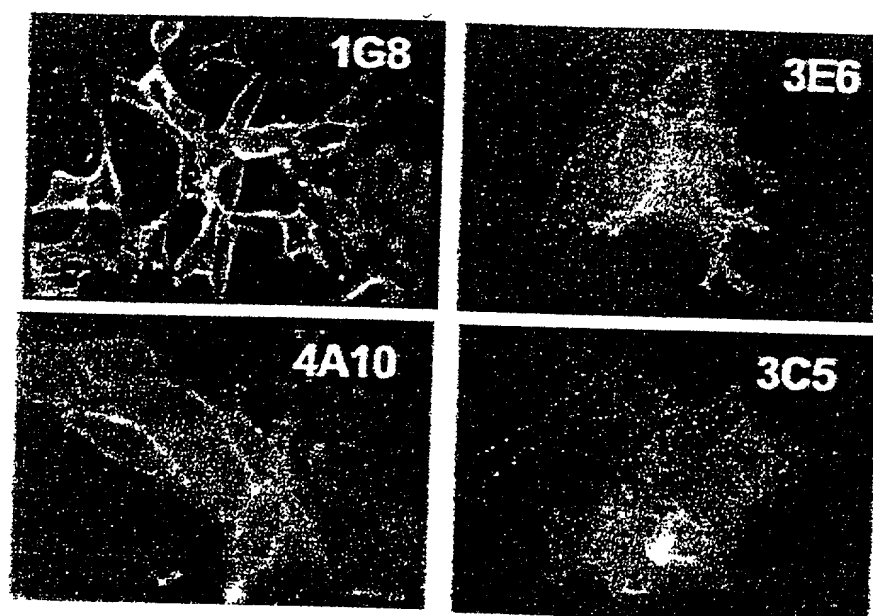


FIGURE 35

TOP SECRET

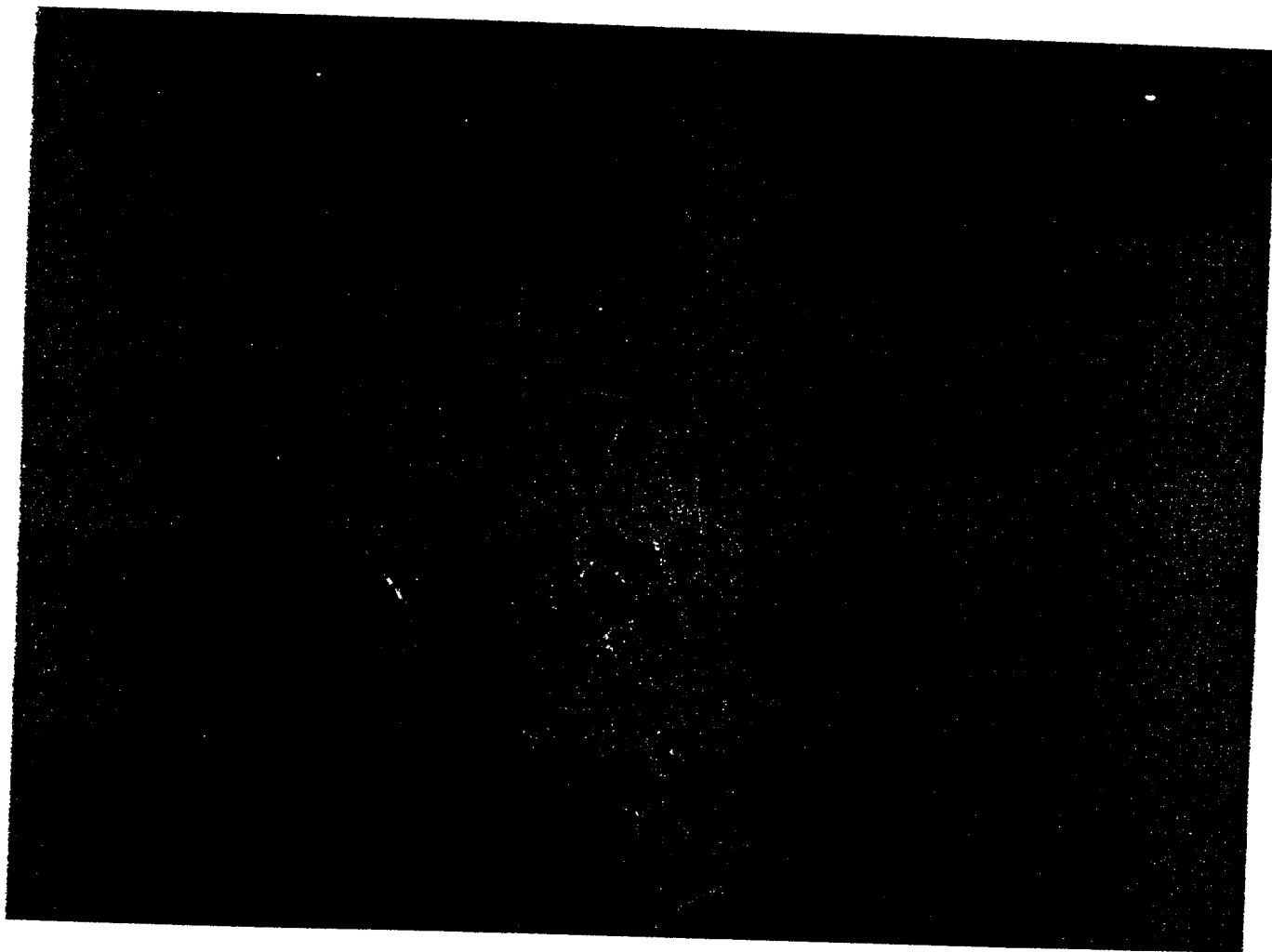


FIGURE 36

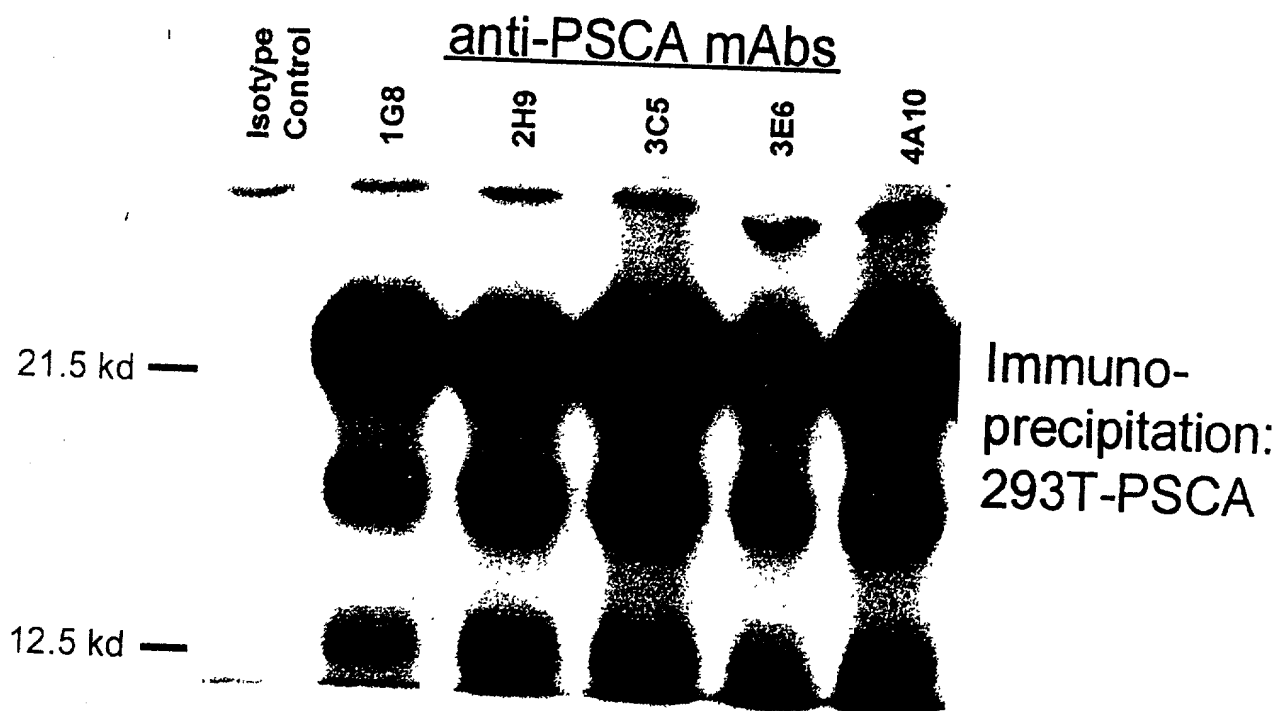


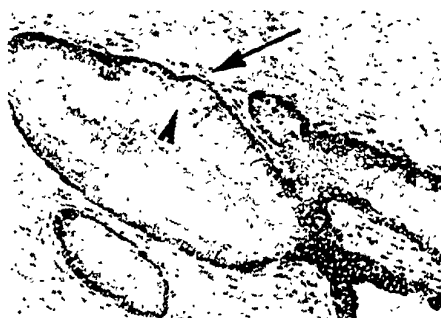
FIGURE 37

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8

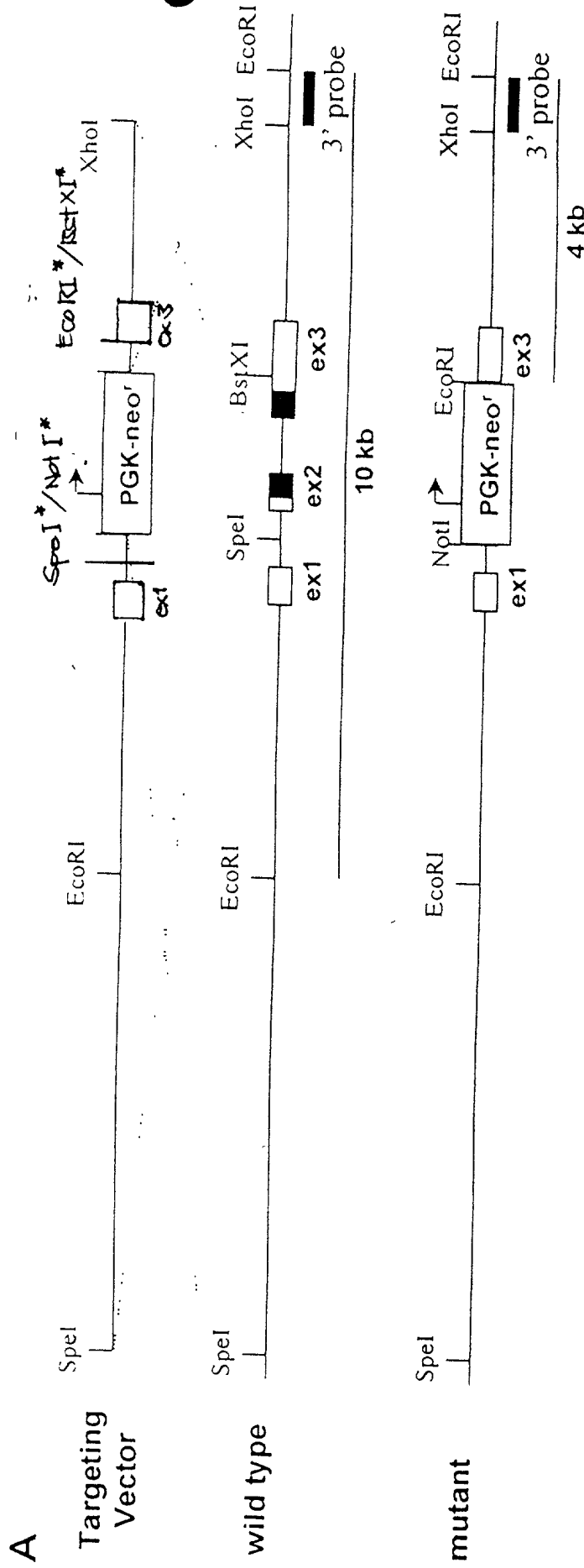


Atrophy: PSCA mAb 2H9



FIGURE 38

Targeting of Mouse PSCA Gene



* ex1, 2, and 3 are the exons of PSCA gene.
 * Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
 * ES genomic DNAs were digested with EcoRI, followed by Southern hybridization using 3' probe.

B. Genomic Southern Analysis of ES Cells

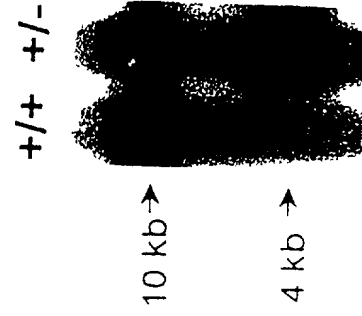
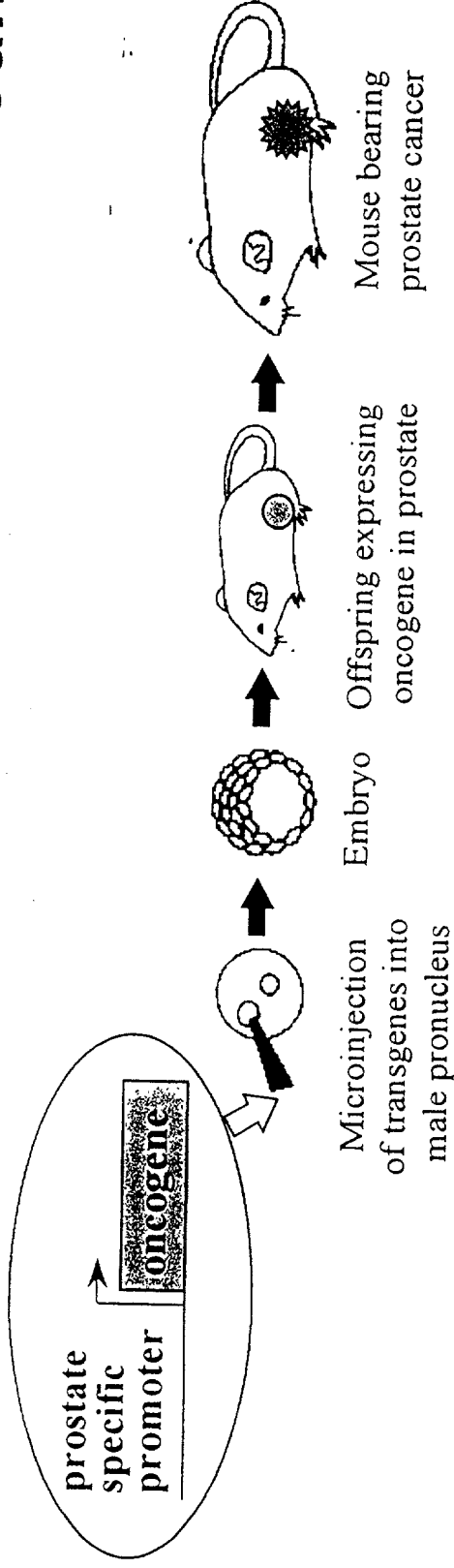


FIGURE 40

Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small T <i>Maroulakou et al.</i> 1994 <i>PNAS</i>	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small T <i>Greenberg et al.</i> 1995 <i>PNAS</i>	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small T <i>Garabedian et al.</i> 1998 <i>PNAS</i>	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 16 wks Metastases in lymph node, lung, liver and bone

FIGURE 41

Reporter Gene Constructs for Transfection Assay

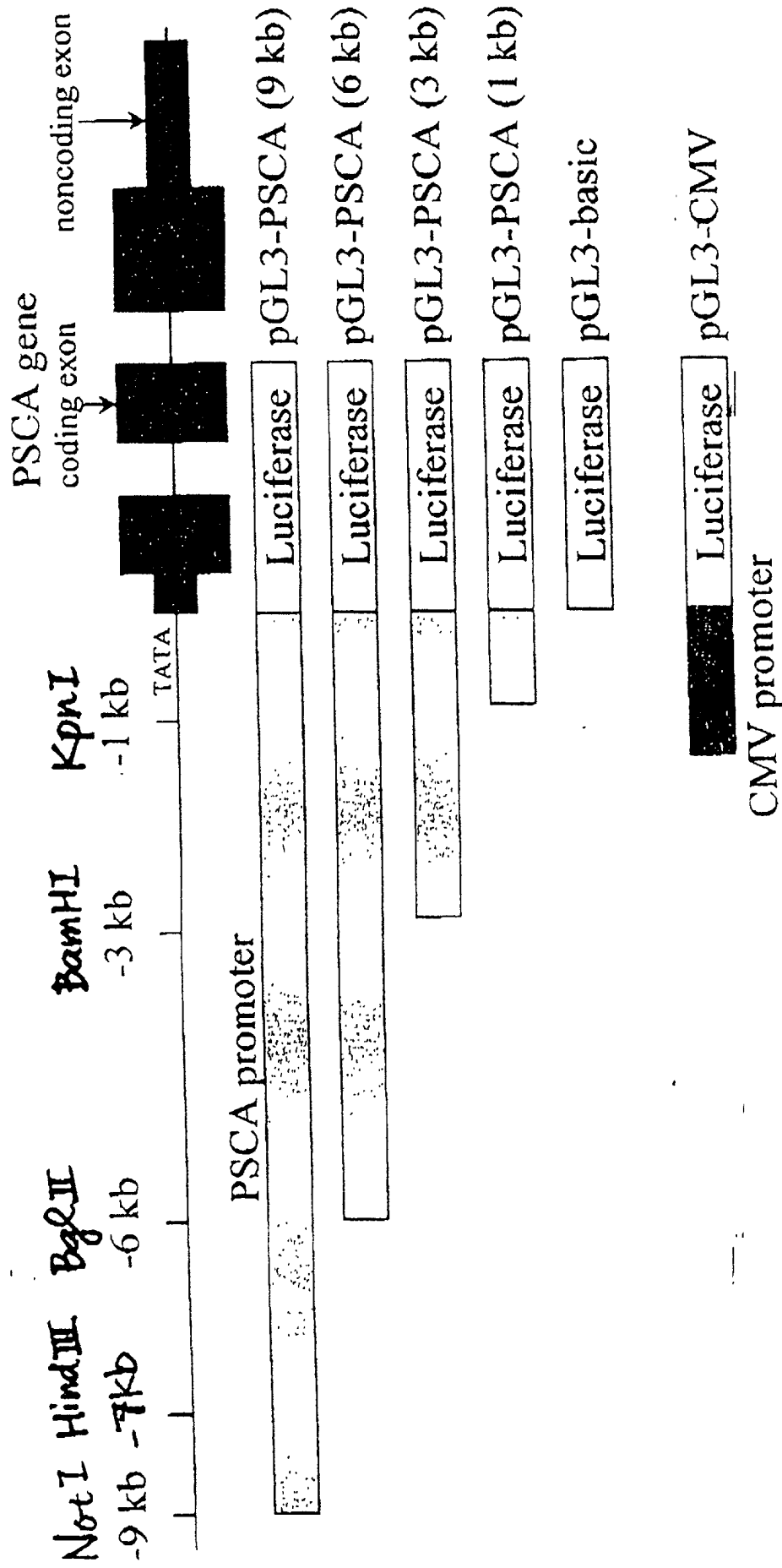


FIGURE 42

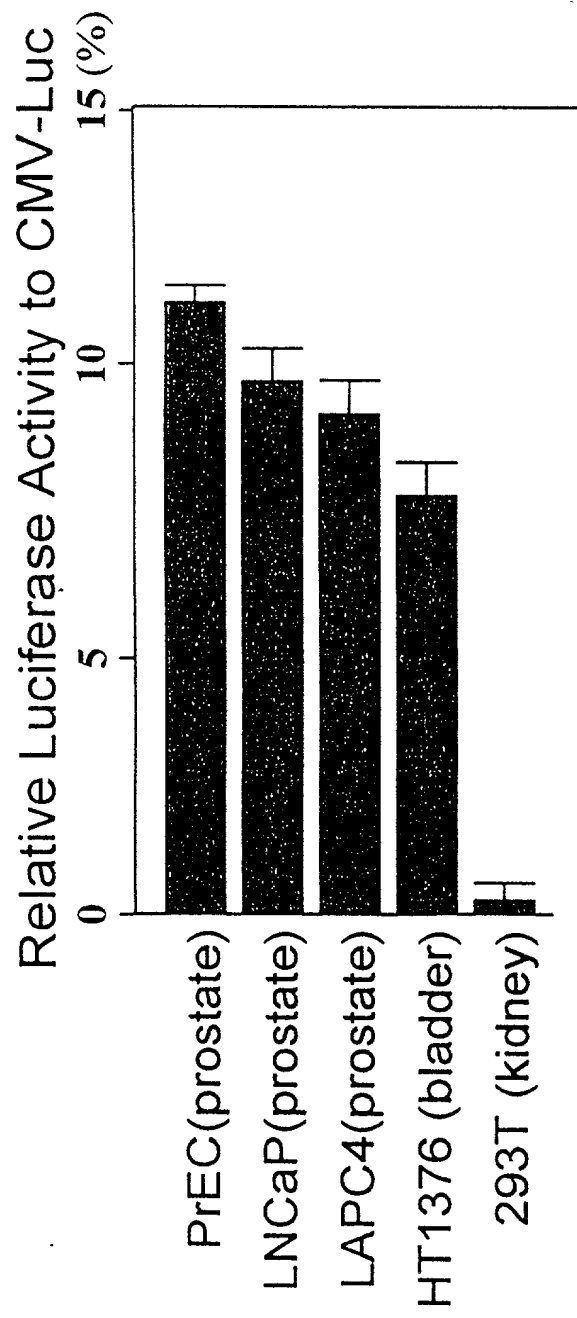


FIGURE 43

Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

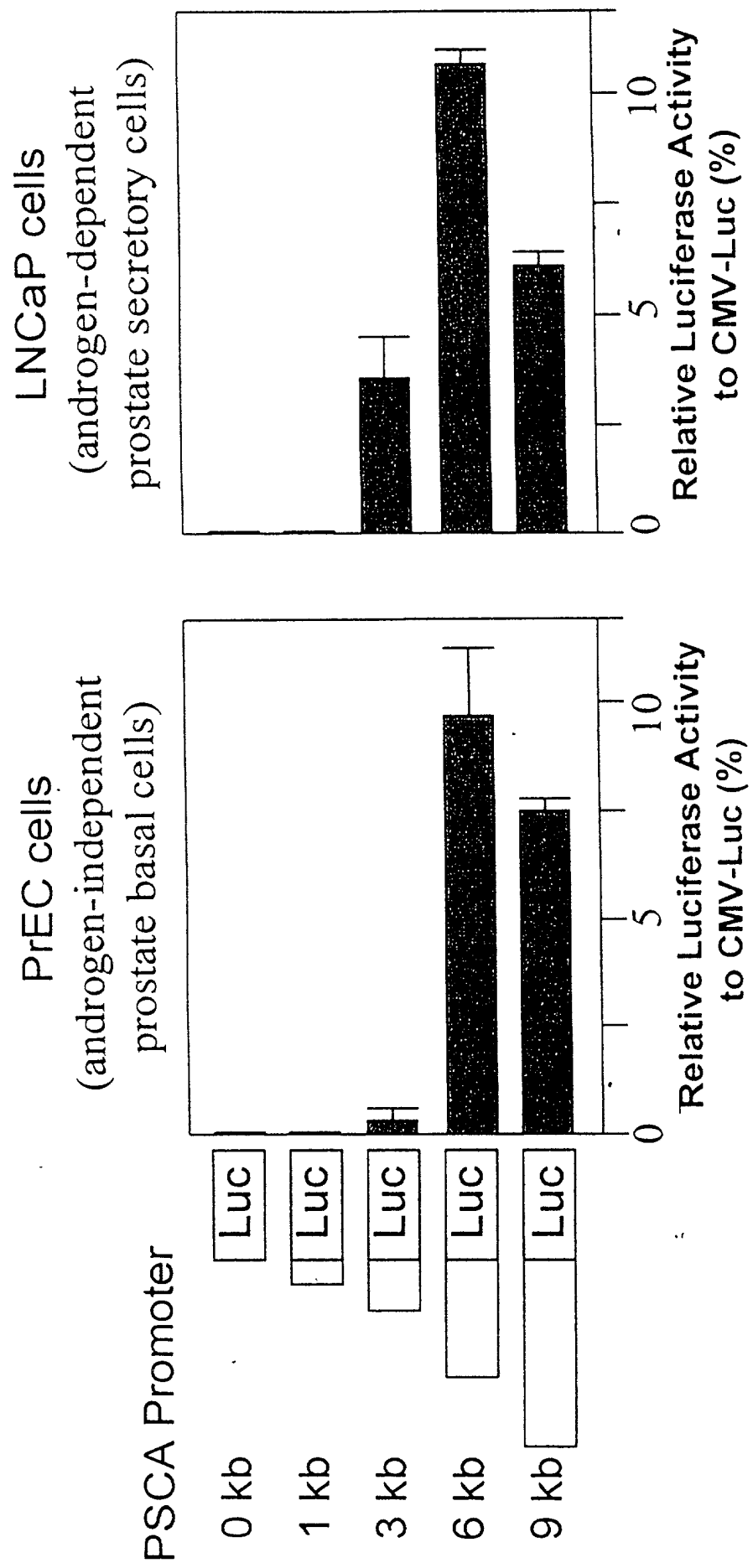


FIGURE 44

Negative tissues

Stomach

Small intestine

Colon

Seminal Vesicle

Urethra

Testis

Liver

Kidney

Lung

Brain

Heart

Skeletal muscle

Ovary

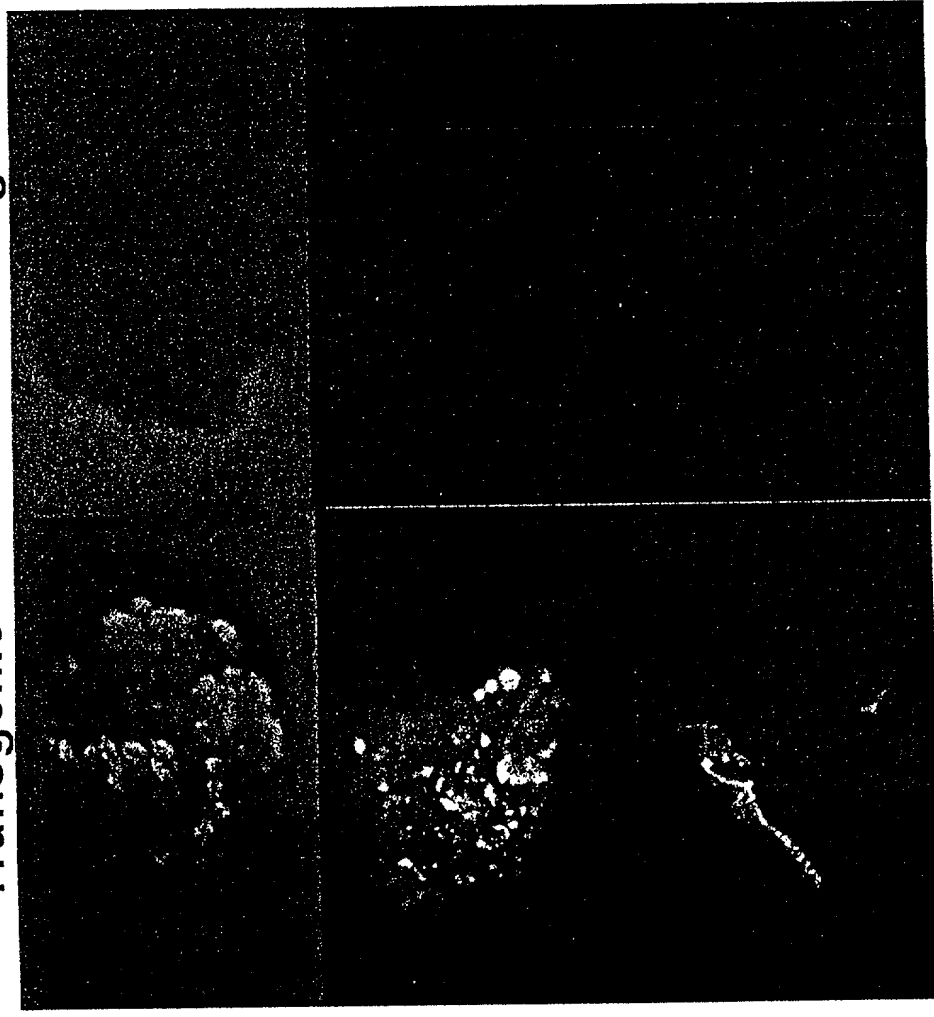
Uterus

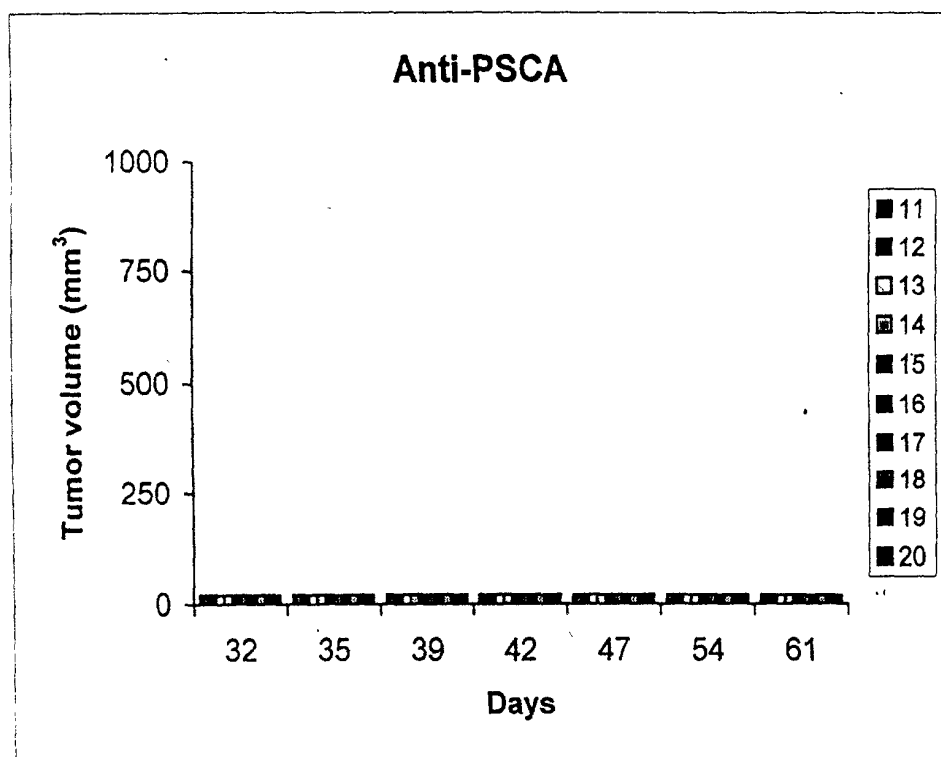
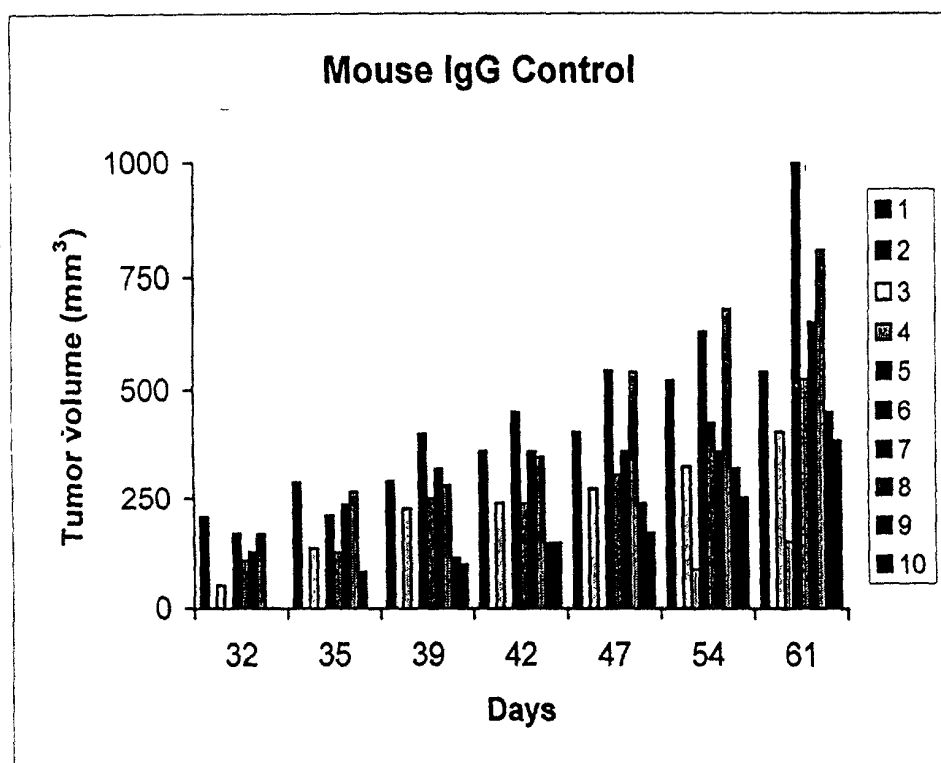
Prostate
(A25-106-2)

Bladder
(A25-104)

Skin
(A25-106-2)

Whole-mount green fluorescence image
Transgenic Non-transgenic



[illegible]

A

FIG. 49

Epitope recognized (OD 450 nm)

mAb	Isotype	F (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

B

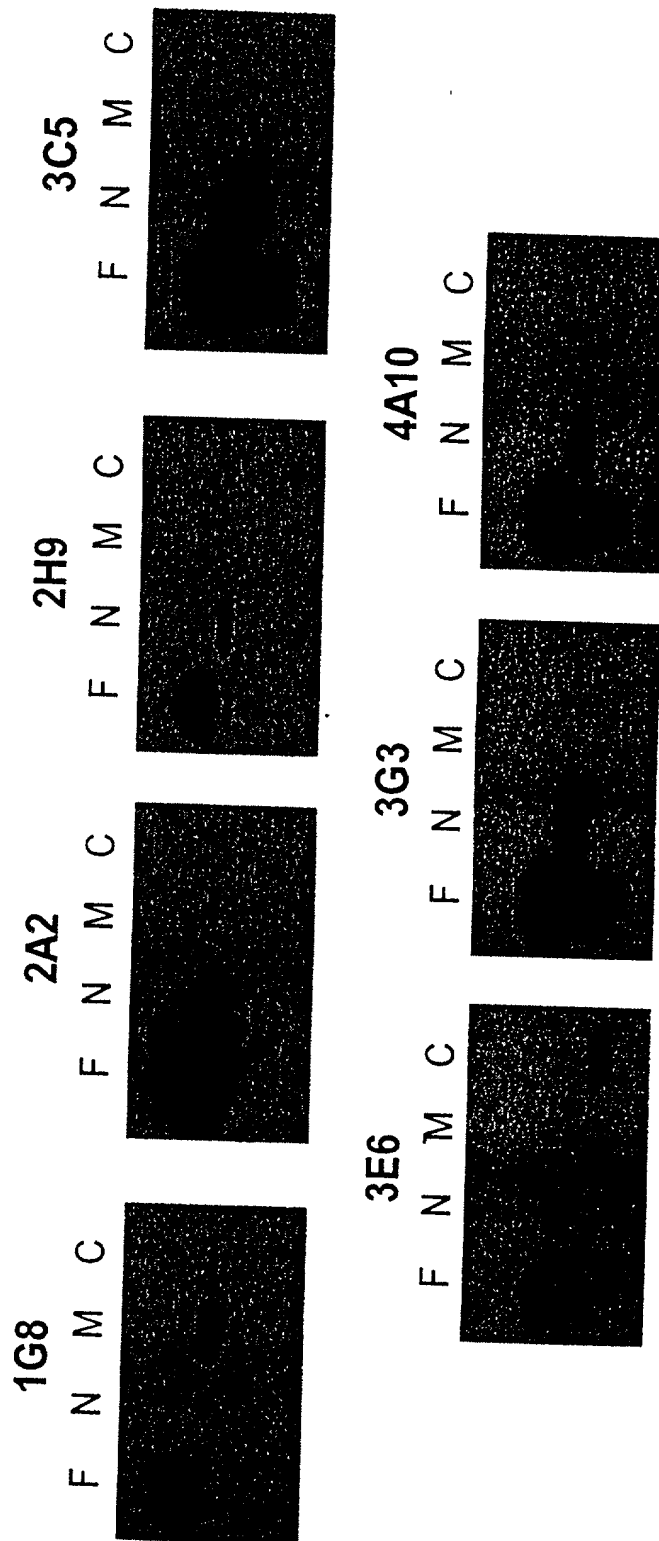
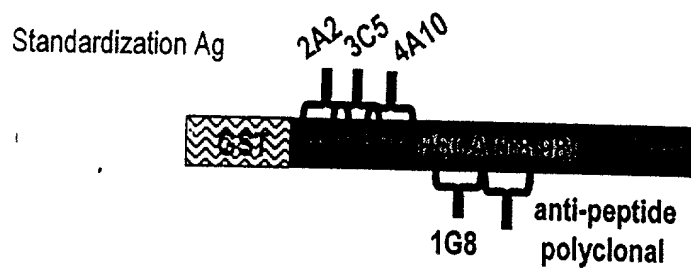
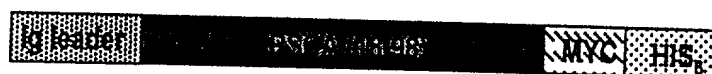


FIG. 50

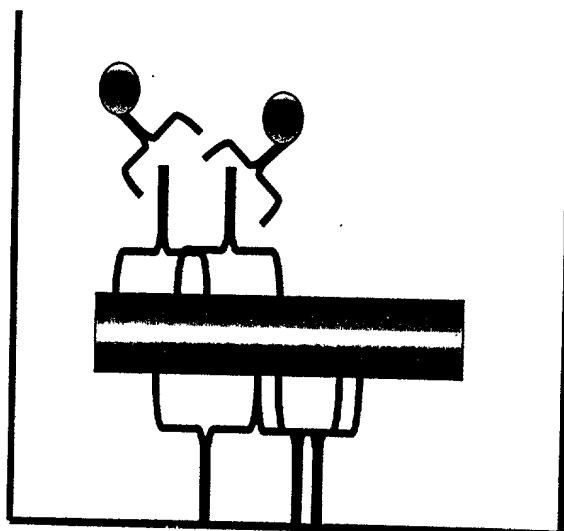
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

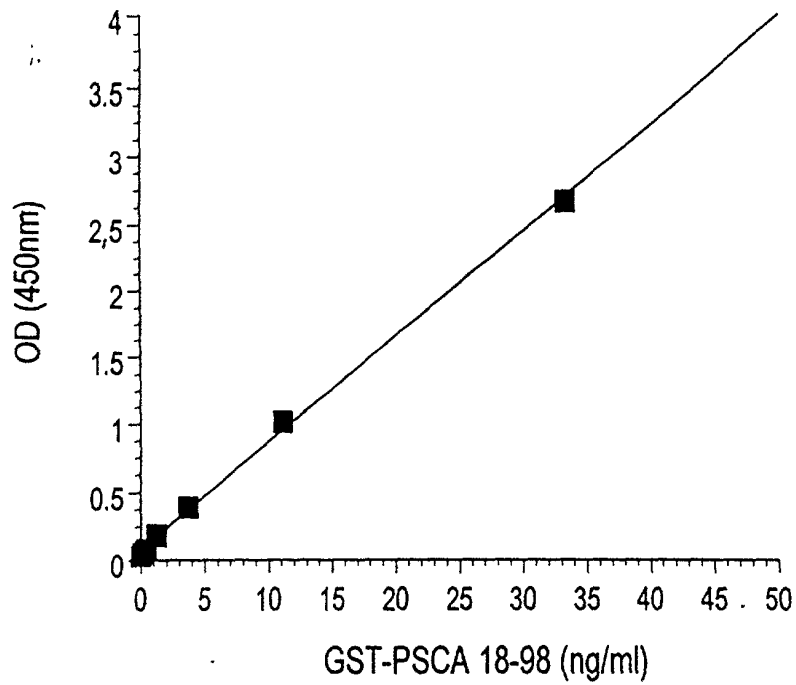
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

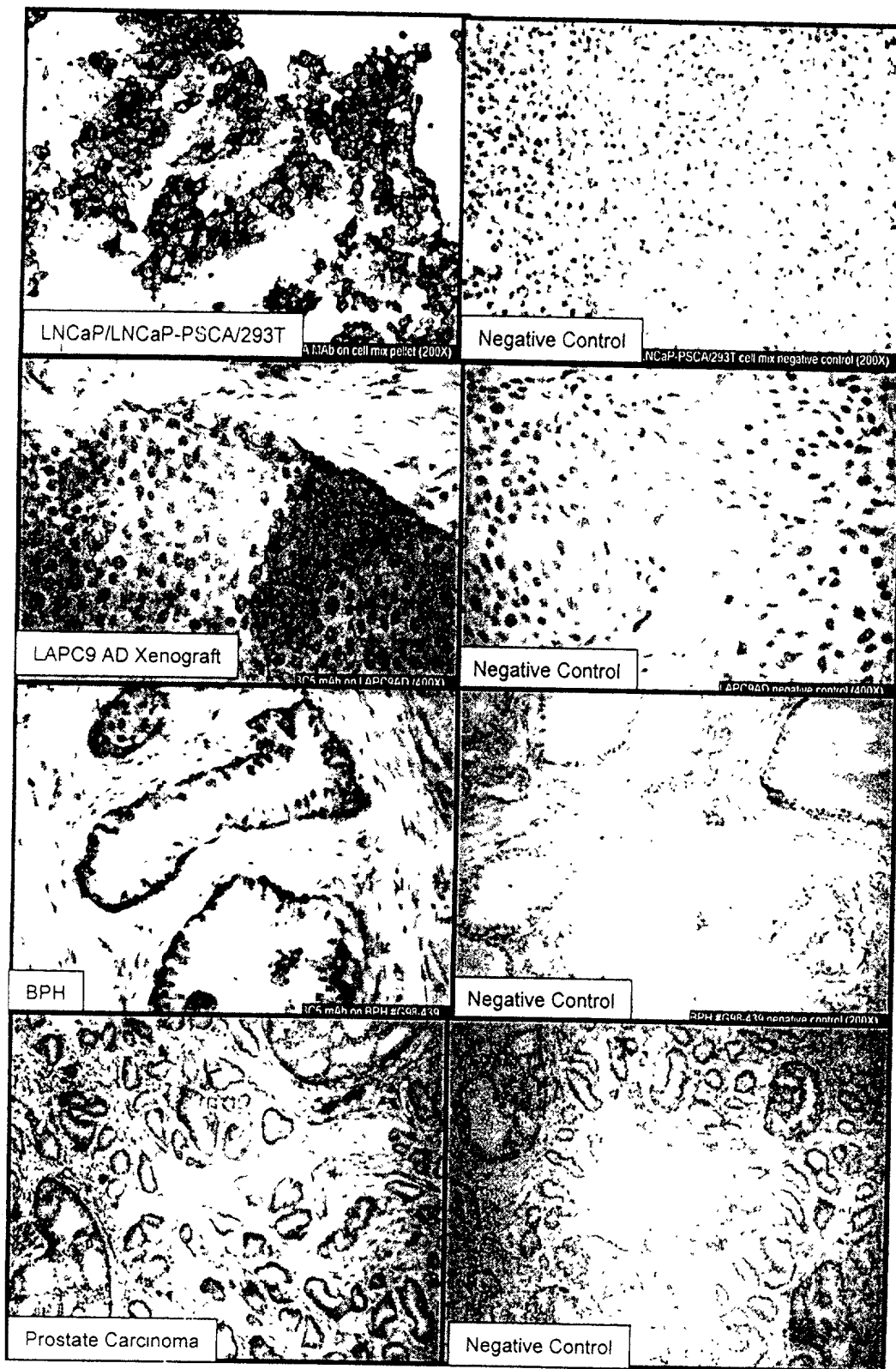


FIG. 53

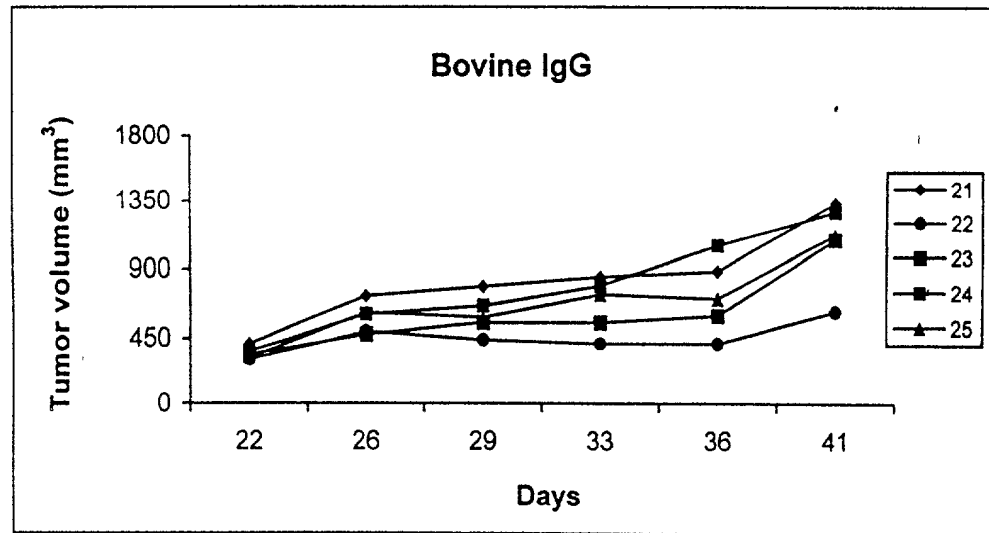
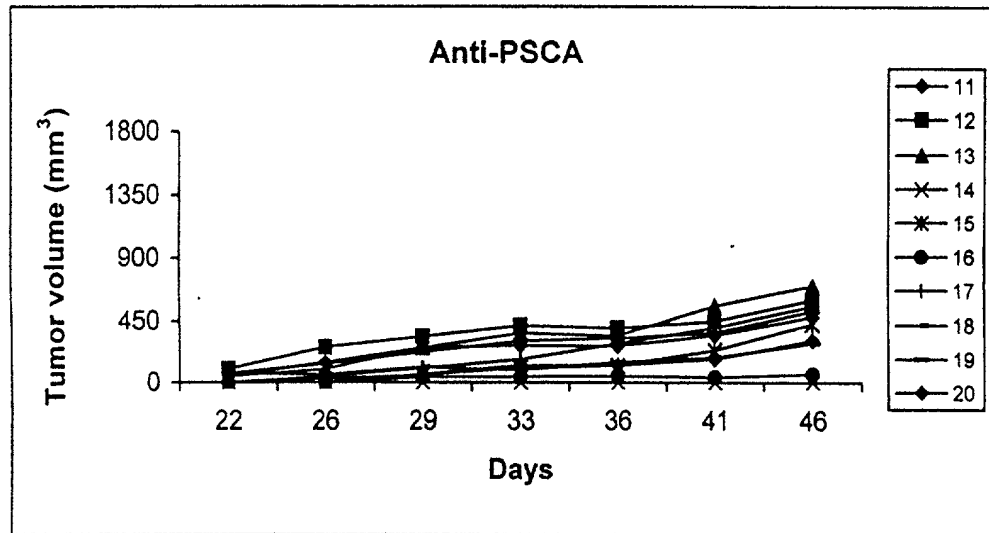
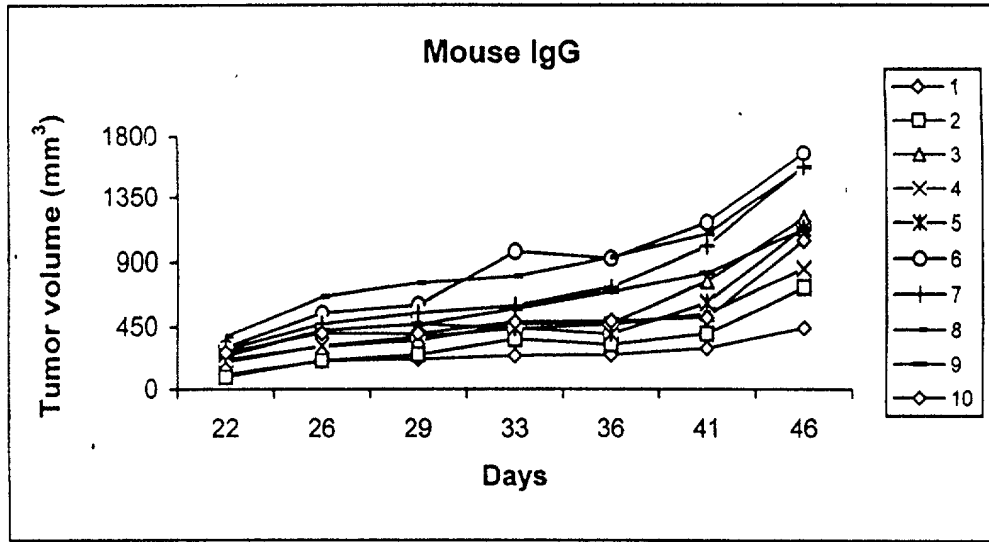


FIG. 54

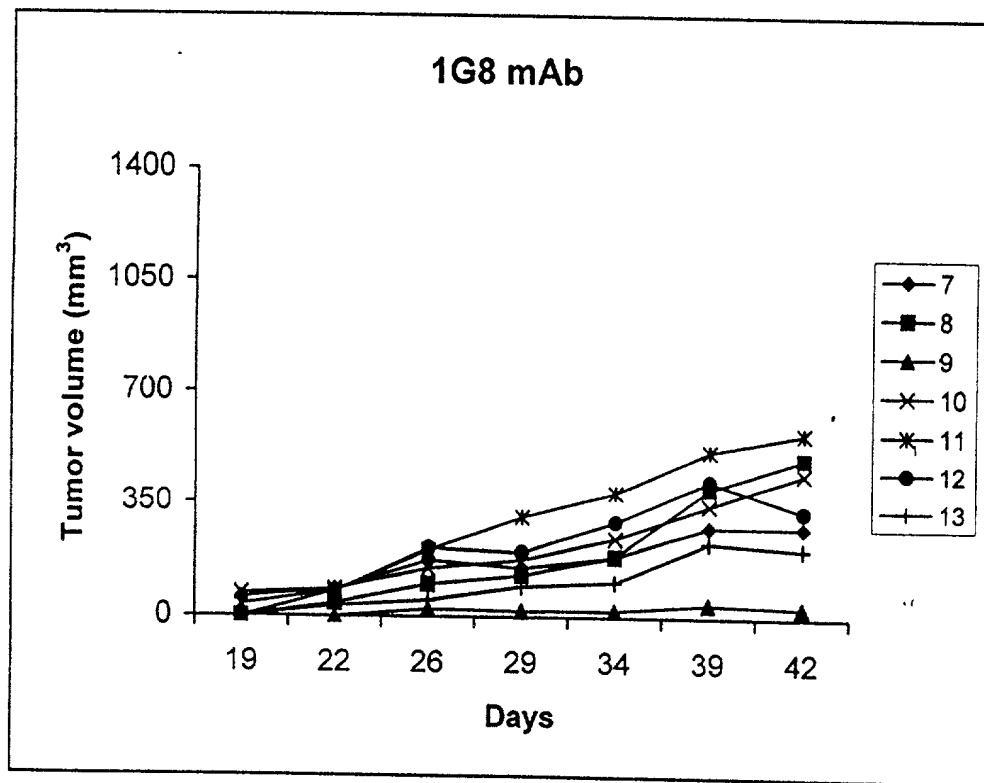
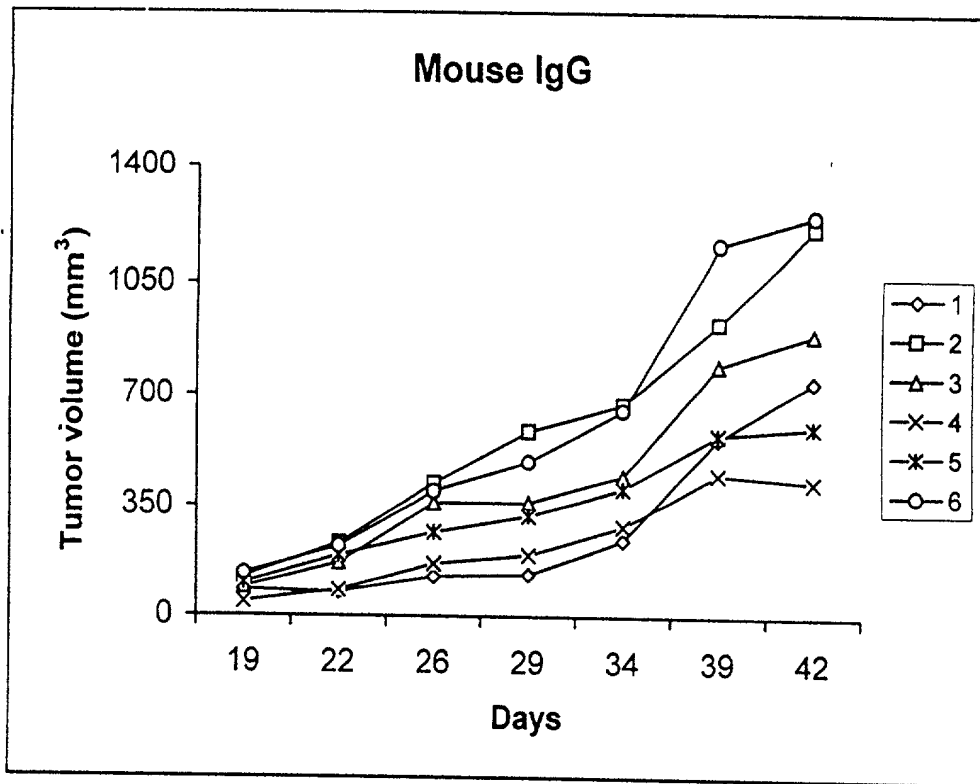


FIG. 55

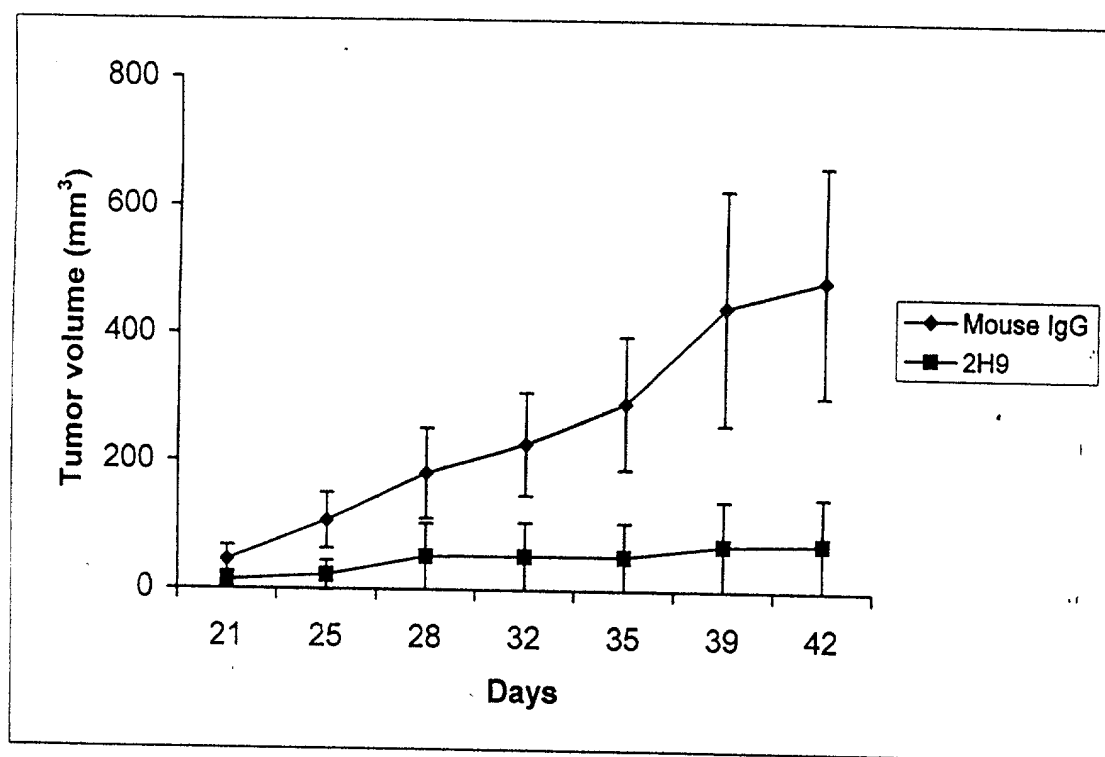
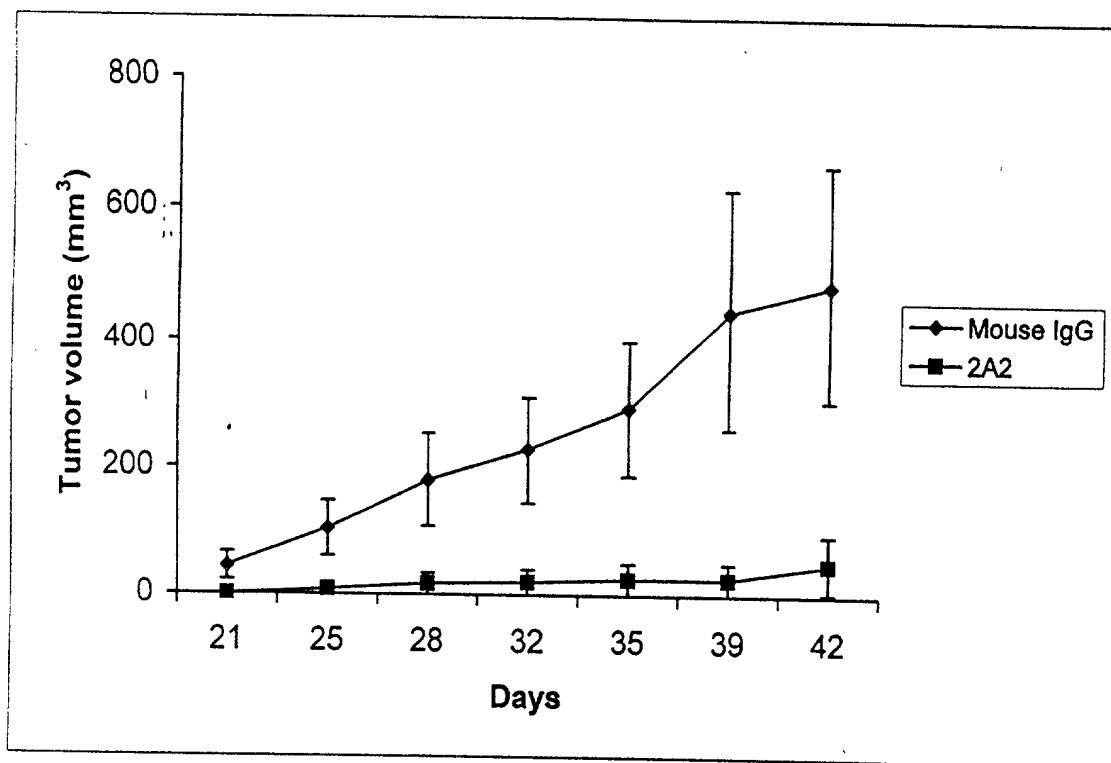


FIG. 56

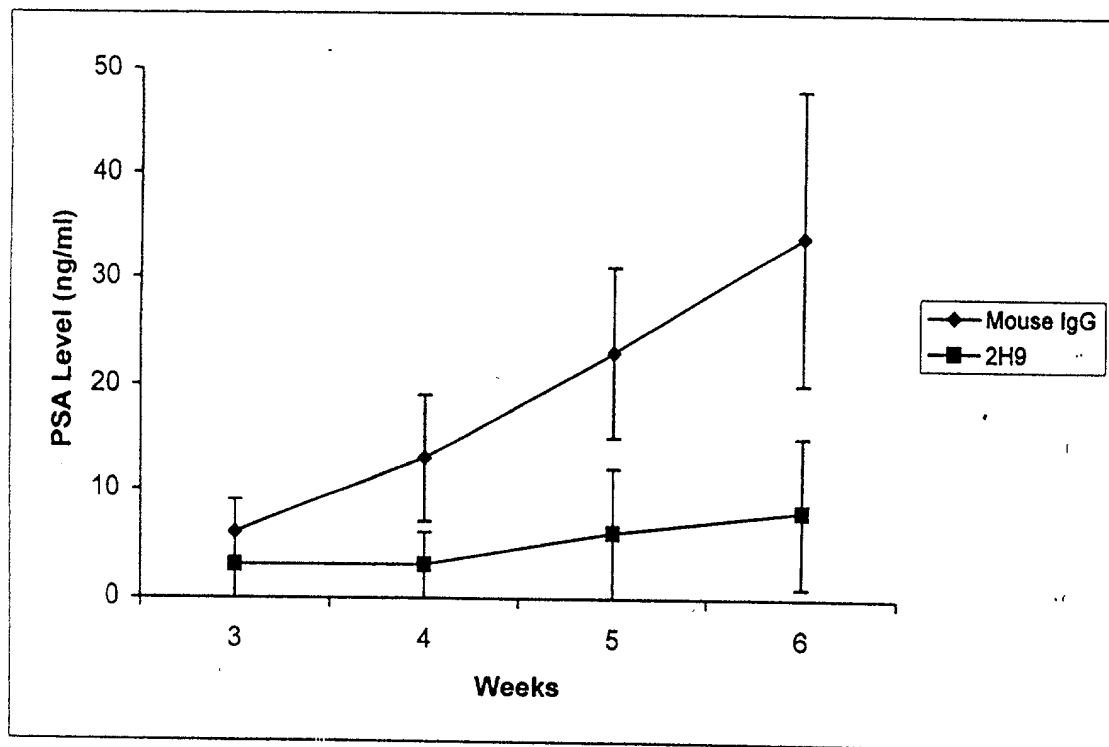
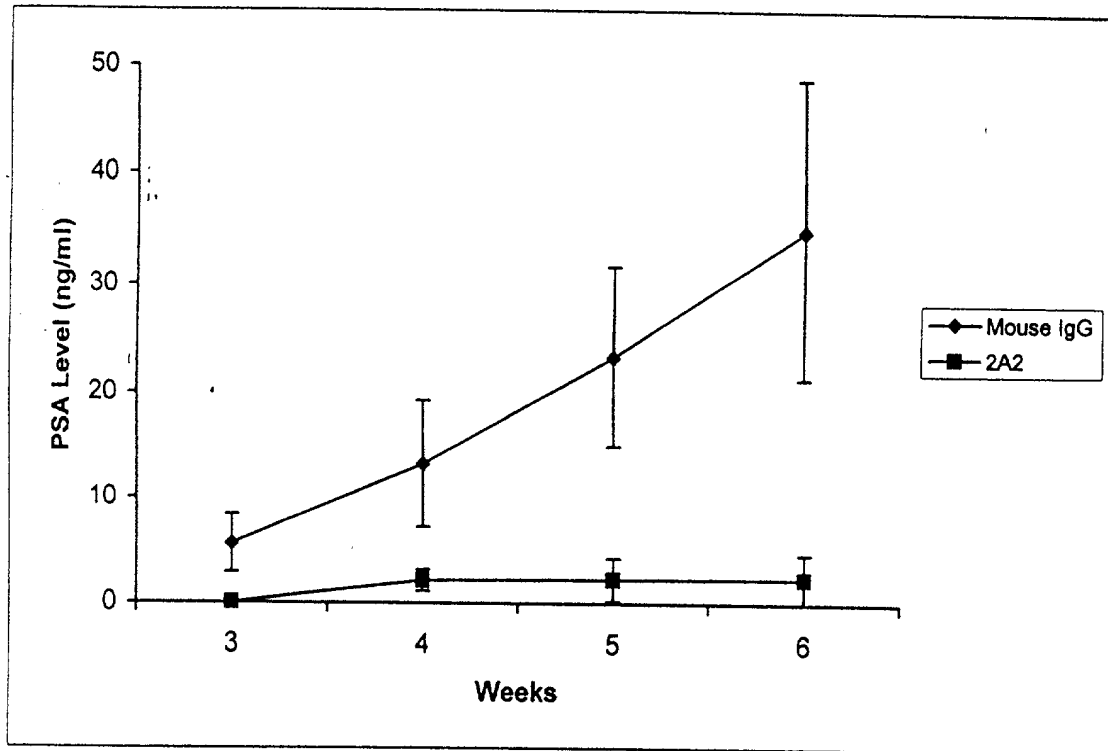


FIG. 57

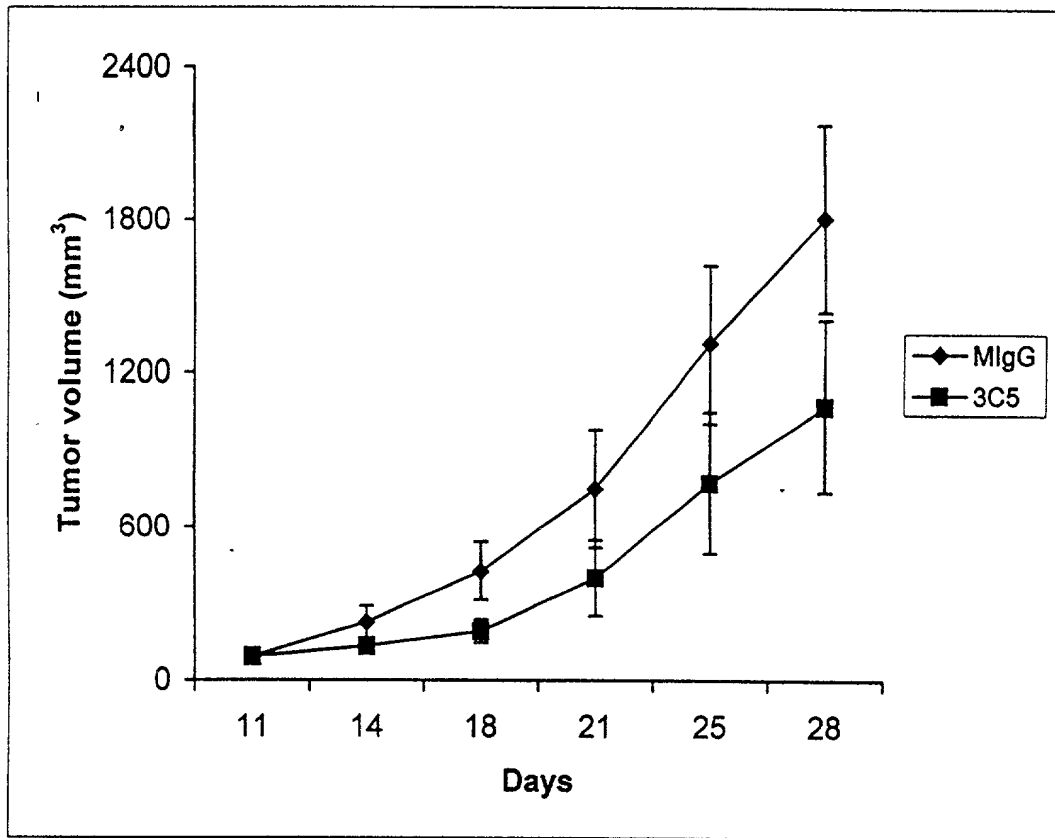


FIG. 58

TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCAATTCAGAGGTTTCAGCTGCAGCAGTCT 60
C F F L M A V V I G V N S E V Q L Q Q S 20

GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120
G A E L V R S G A S V K L S C T A S G F 40

CDR1
AACATTAAAGACTACTATATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180
N I K D Y Y I H W V N Q R P D Q G L E W 60

CDR2
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240
I G W I D P E N G D T E F V P K F O G K 80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
A T M T A D I F S N T A Y L H L S S L T 100

CDR3
TCTGAAGACACTGCCGTCTATTACTGTAAACGGGGGTTTCTGGGGCCAAGGGACTCTG 360
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG
V T V S A A K T T P P S V Y P L

FIG. 58

FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAGTGCAGCAACCTGGGTCTGAA 60
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAAGTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120
L V R P G T S V K L S C K A S G Y T F S 40
CDR1

AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
I D P G S G Y T N Y A E N L K T K A T L 80
CDR2

ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTTGCTTACTGG 360
S A V Y Y C T S R S T M I T T G F A Y W 120
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC
L A

FIG. 59 "ESTS" 40

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTTATTATTGTTCTTTTAAAAGGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTTACTTTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1

GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGCGGAGTCTGTGAAAGGGAAATTCACCATCTCAAGAGATGATTCCAGAAGTCGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGAATTTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCCATCTGTCTATCCACTGGCCCCTTGTGTA
P P S V Y P L A P C V

FIG. 60 " CDR1 CDR2 CDR3

FIG. 61

CDR1 Comparisons

1G8	1gG _{1k}	Middle	G	F	N	I	K	D	Y	Y	I	H
2H9	1gG _{1k}	N-Term.	G	F	T	F	S	N	Y	W	M	T
4A10	1gG _{2ak}	N-Term.	G	Y	T	F	S	S	Y	W	M	H

CDR2 Comparisons

1G8	1gG _{1k}	W	I	D	P	E	N	G	D	T	E	F	V	P	K	F	Q	G		
2H9	1gG _{1k}	E	I	R	L	R	S	E	N	Y	A	T	H	Y	A	E	S	V	K	G
4A10	1gG _{2ak}	N	I	D	P	G	S	G	Y	T	N	Y	A	E	N	L	K	T		

CDR3 Comparisons

1G8	1gG _{1k}	G	G	F								
2H9	1gG _{1k}	L	G	R	P	N						
4A10	1gG _{2ak}	R	S	T	M	I	T	T	G	F	A	Y

FIG. 62

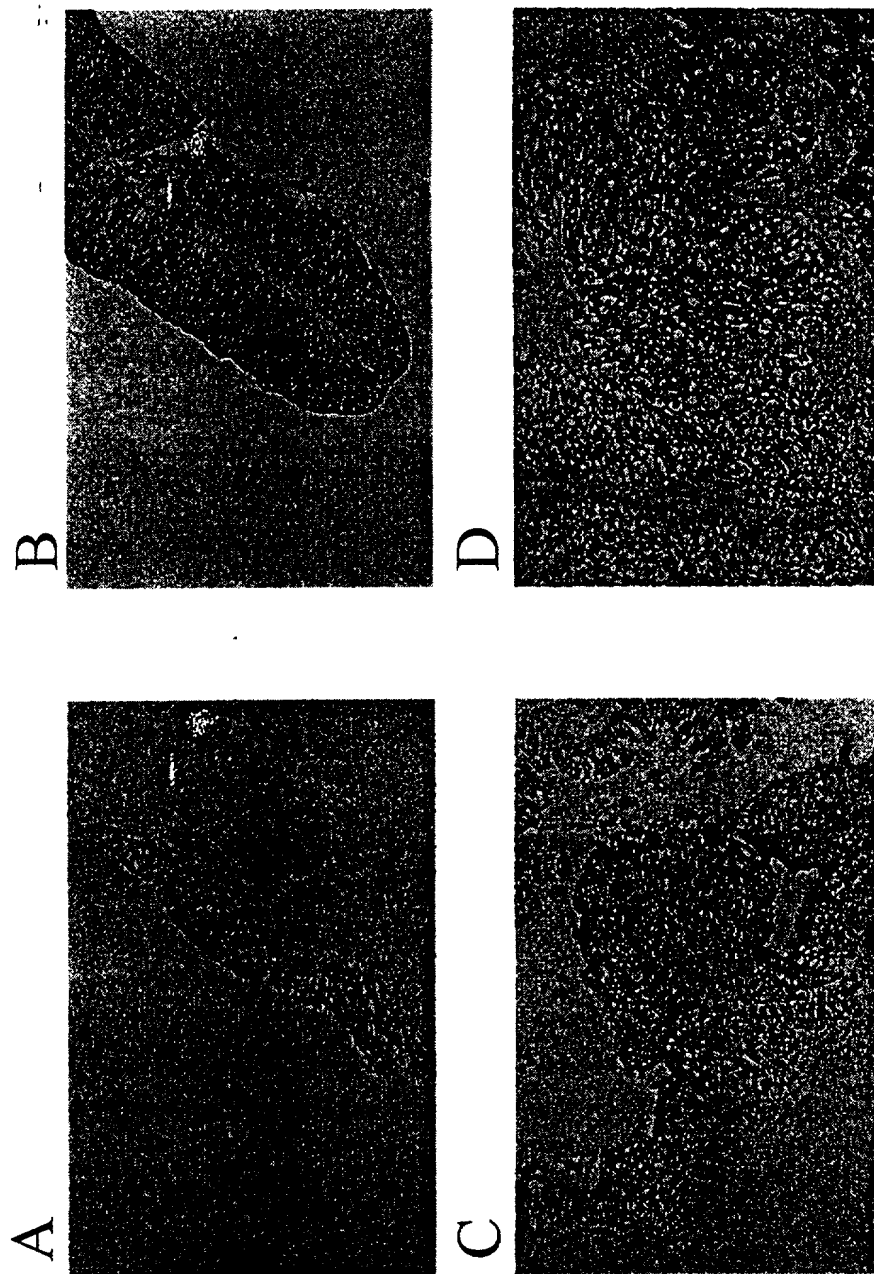


FIG. 63

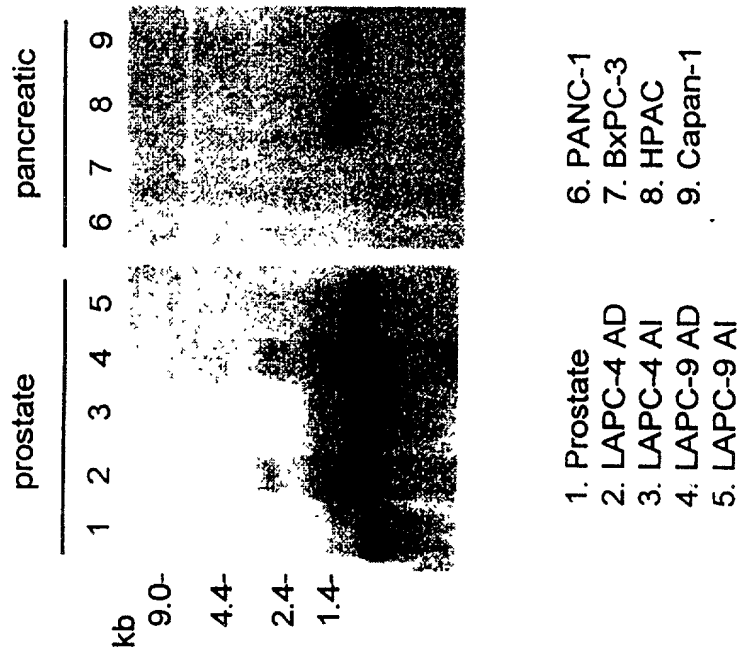
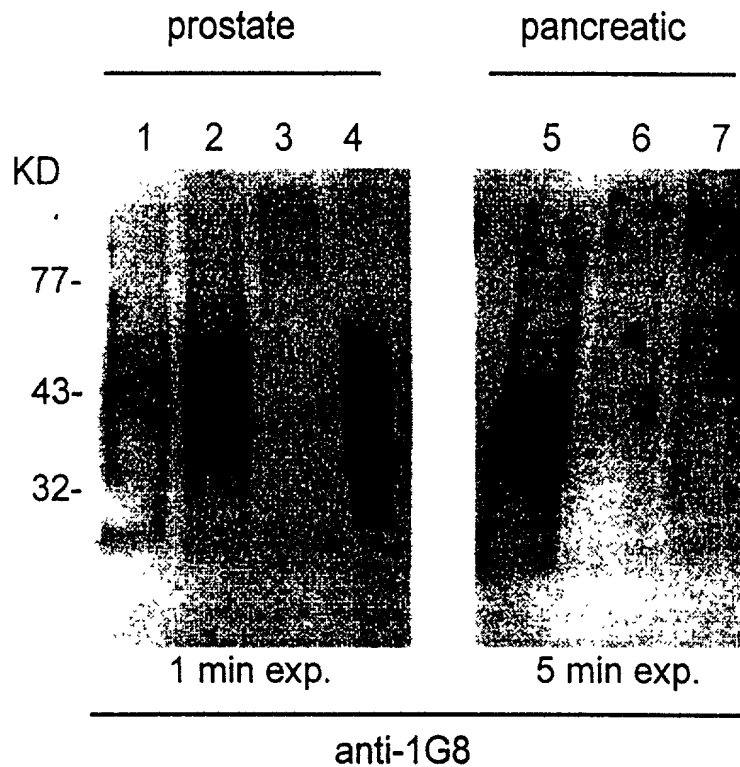


FIG. 64



- 1. LAPC-4 AD
- 2. LAPC-9 AI
- 3. LNCaP
- 4. LNCaP-PSCA

- 5. HPAC
- 6. Capan-1
- 7. ASPC-1

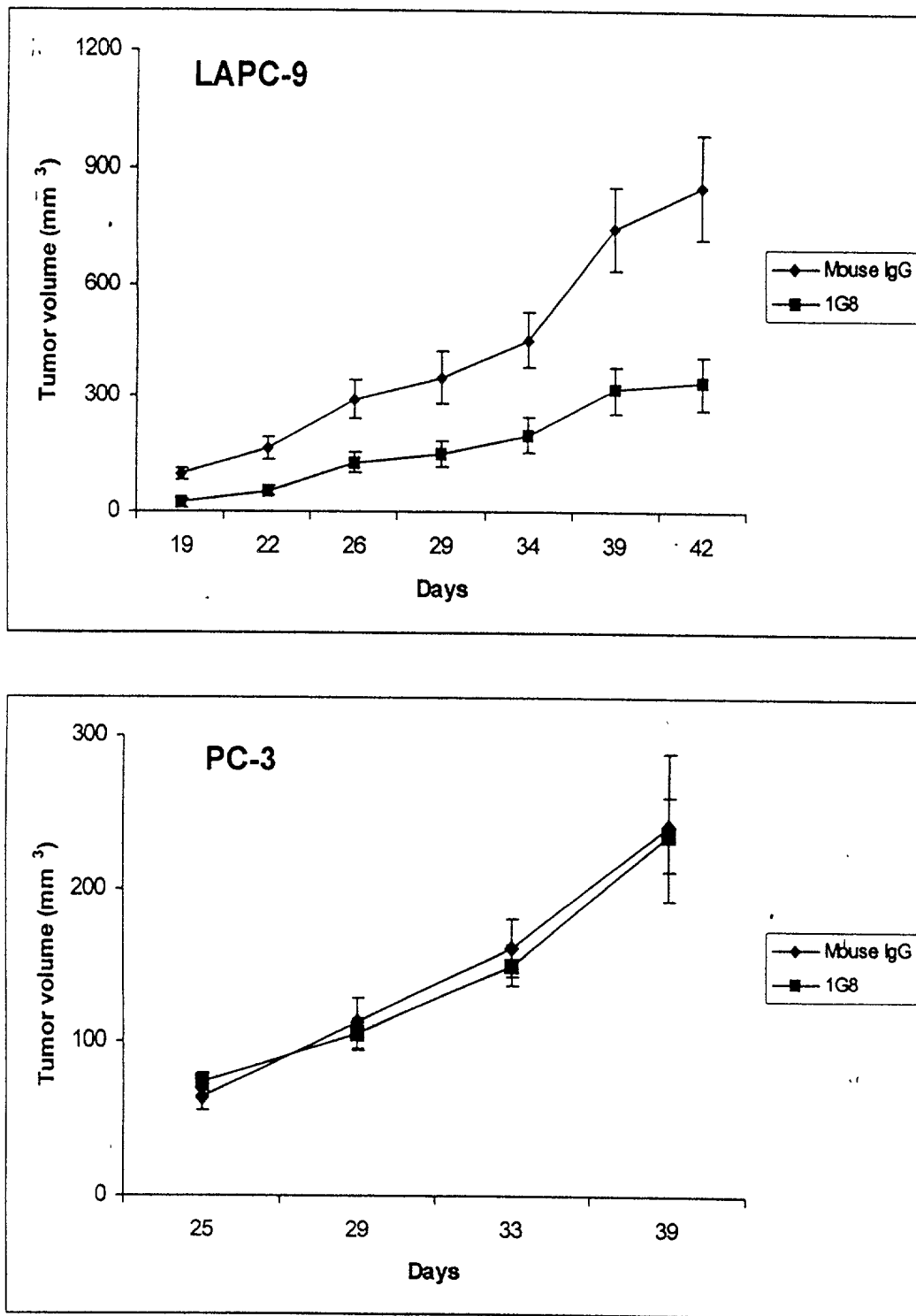
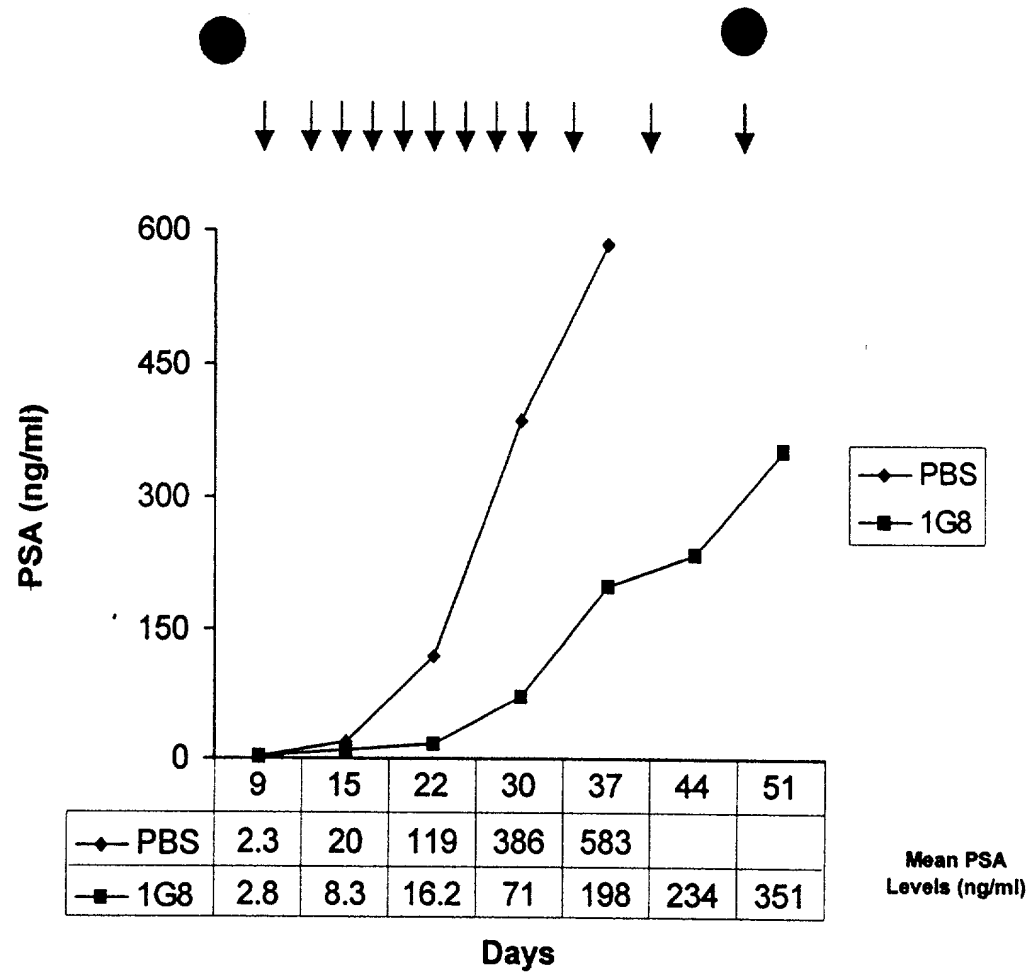


FIGURE 65

TOP SECRET

A)



B)

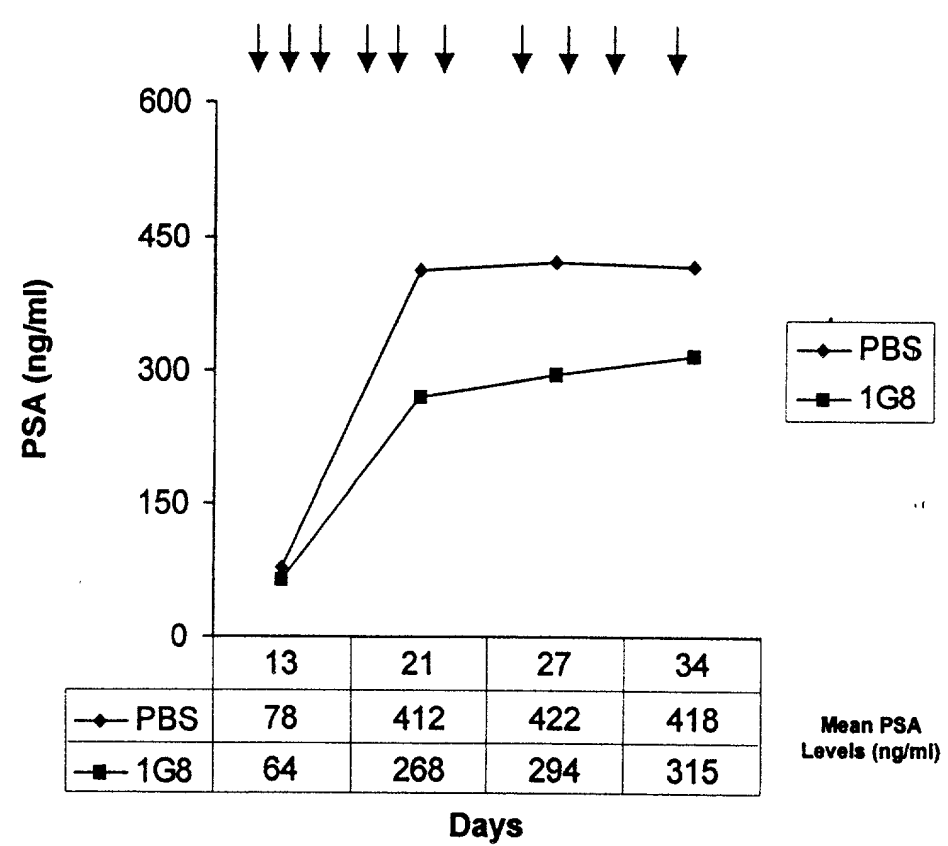
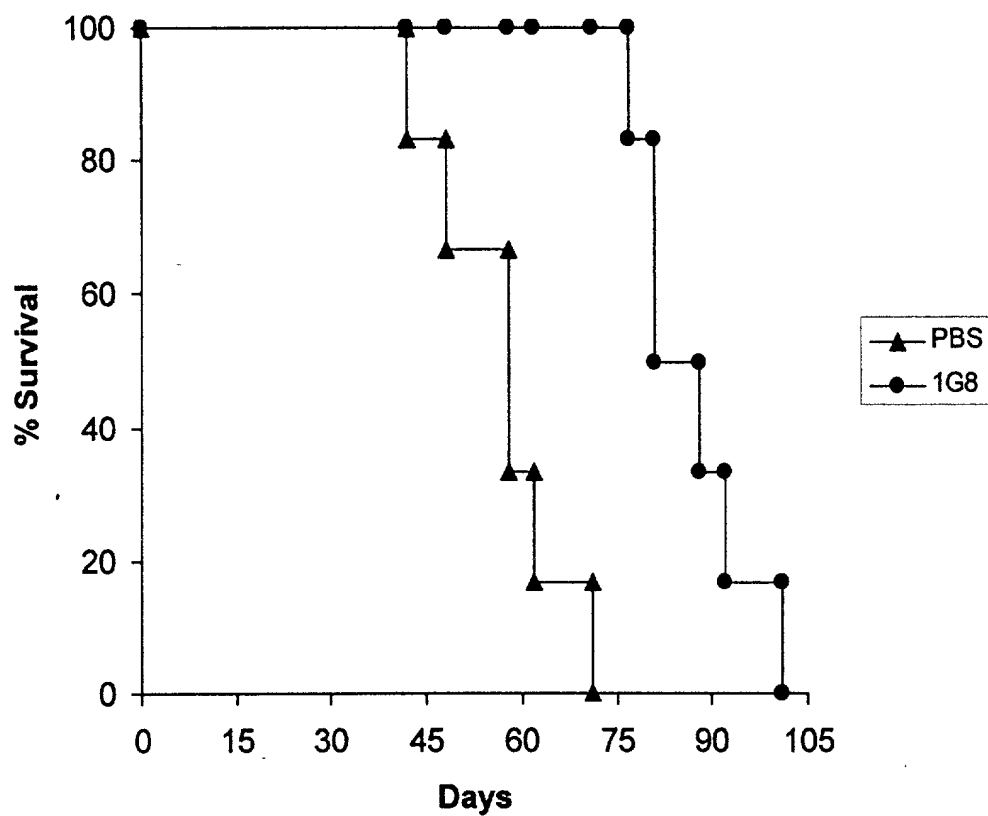


Figure 66

A)



B)

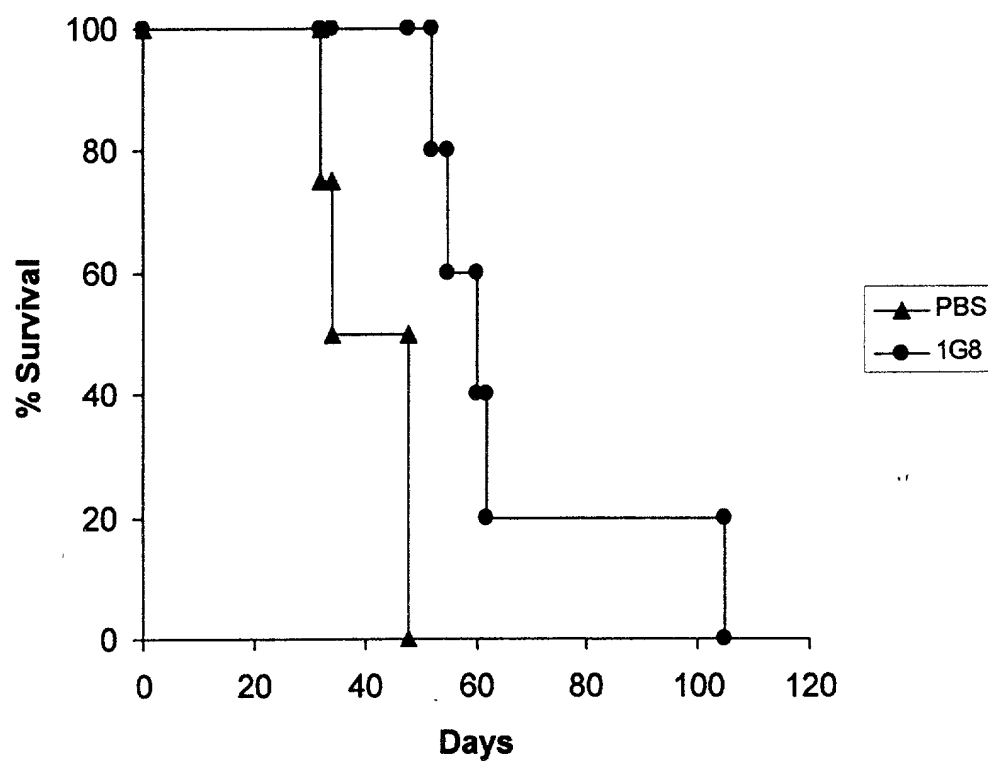
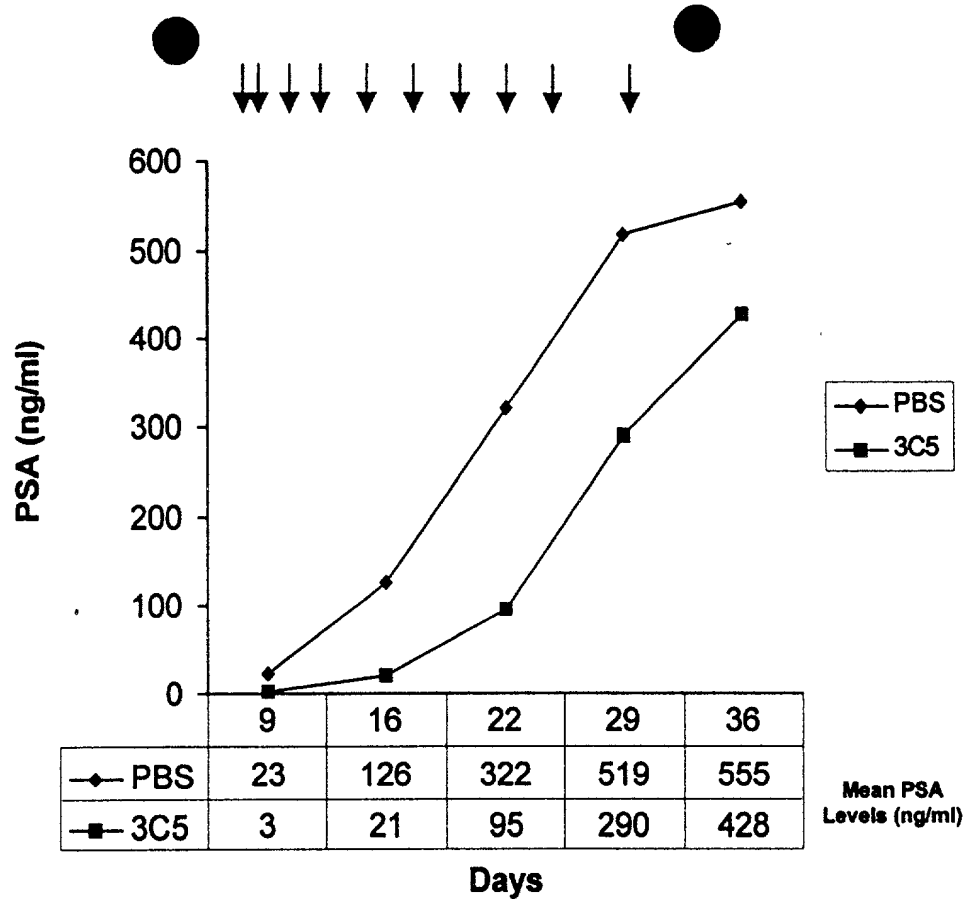


Figure 67

A)



B)

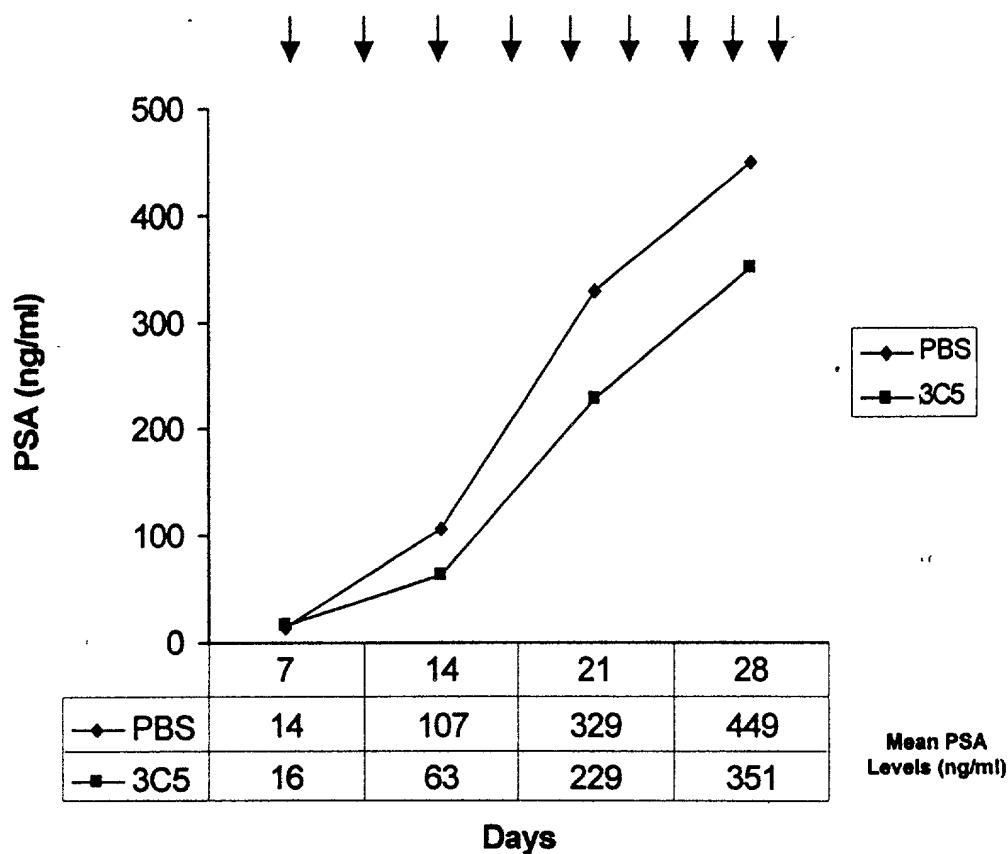
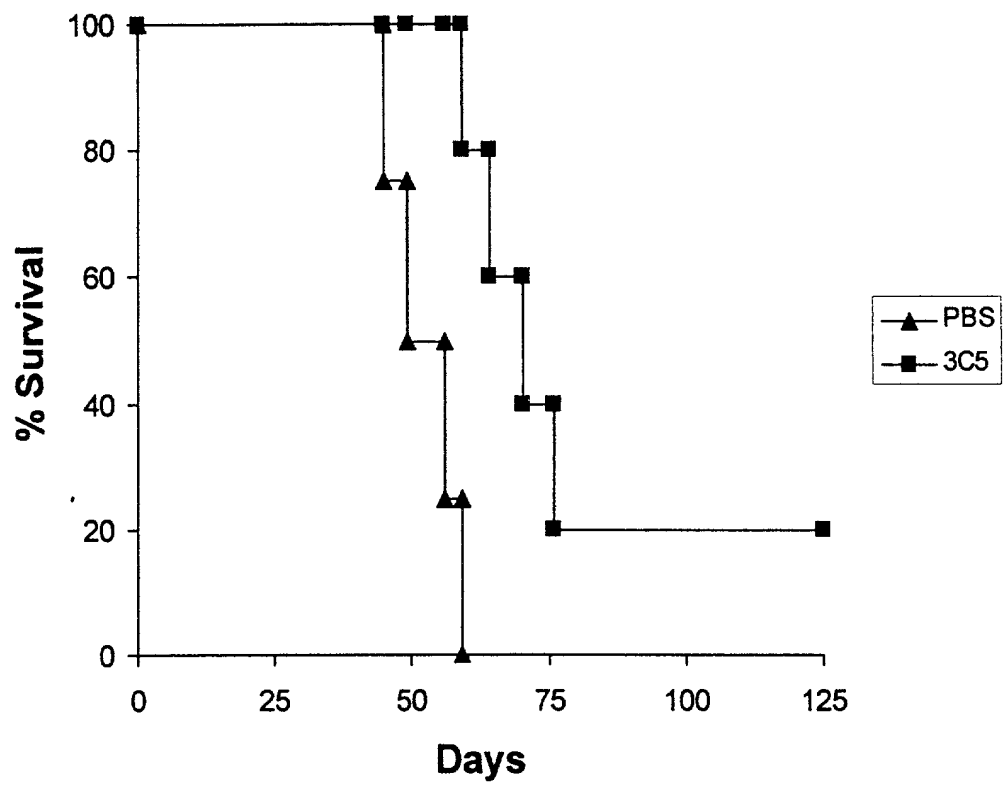


Figure 68

A)



B)

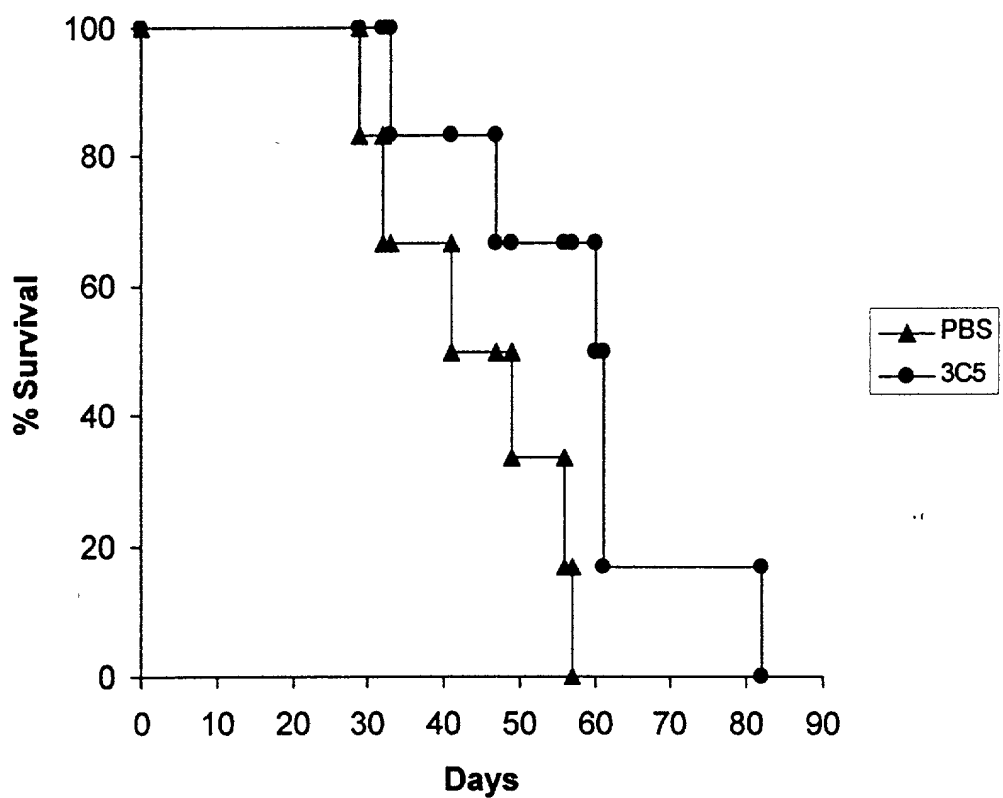


Figure 69

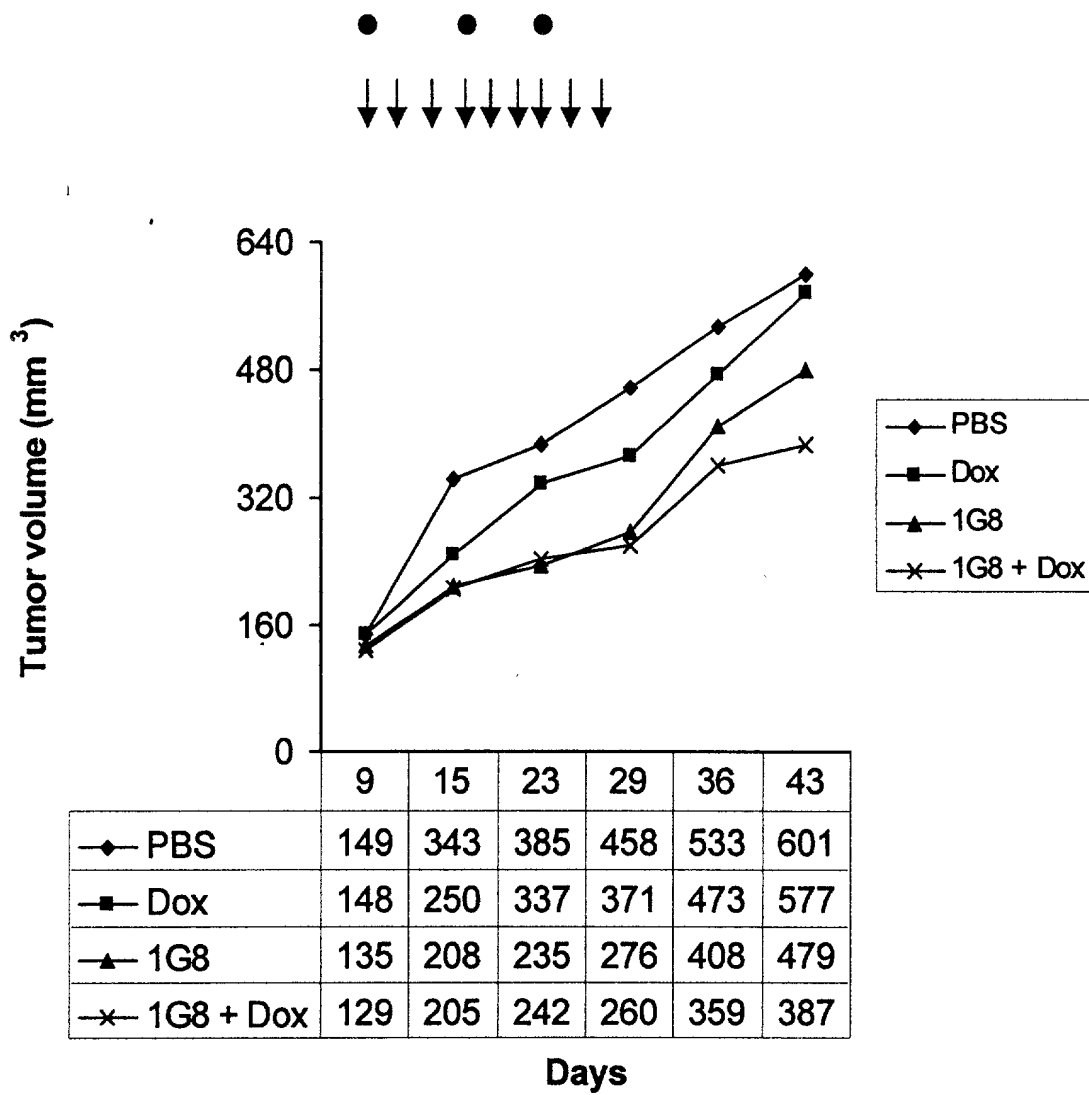
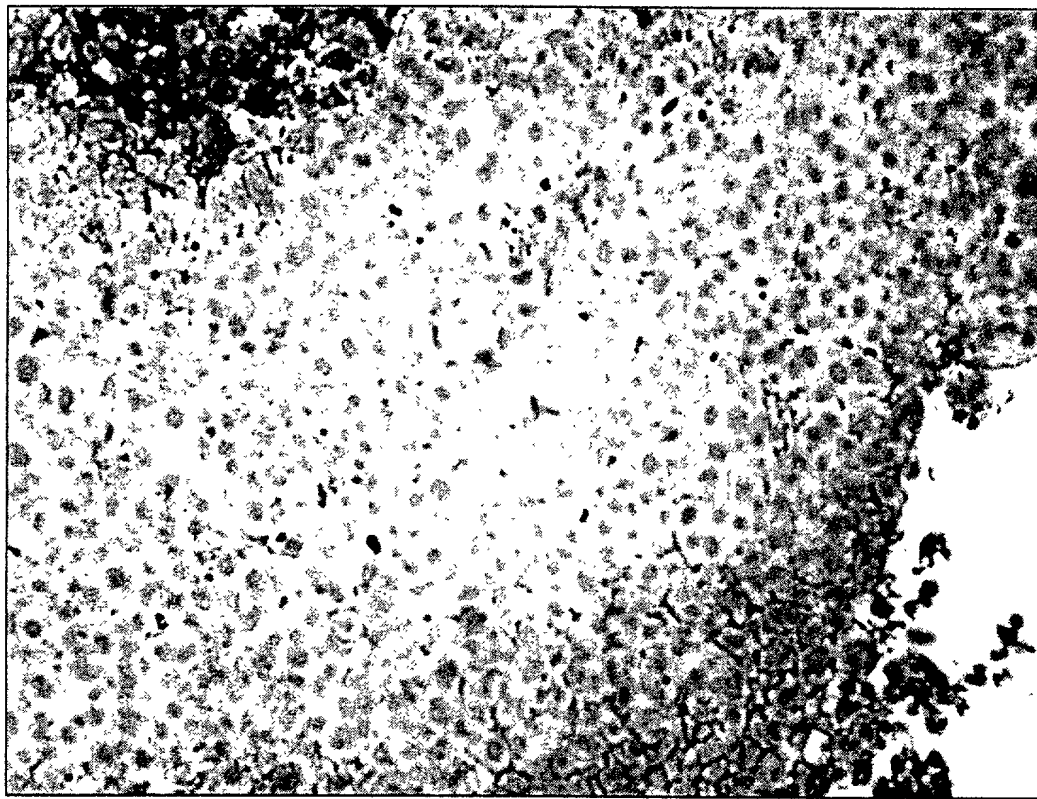


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mIgG Treated

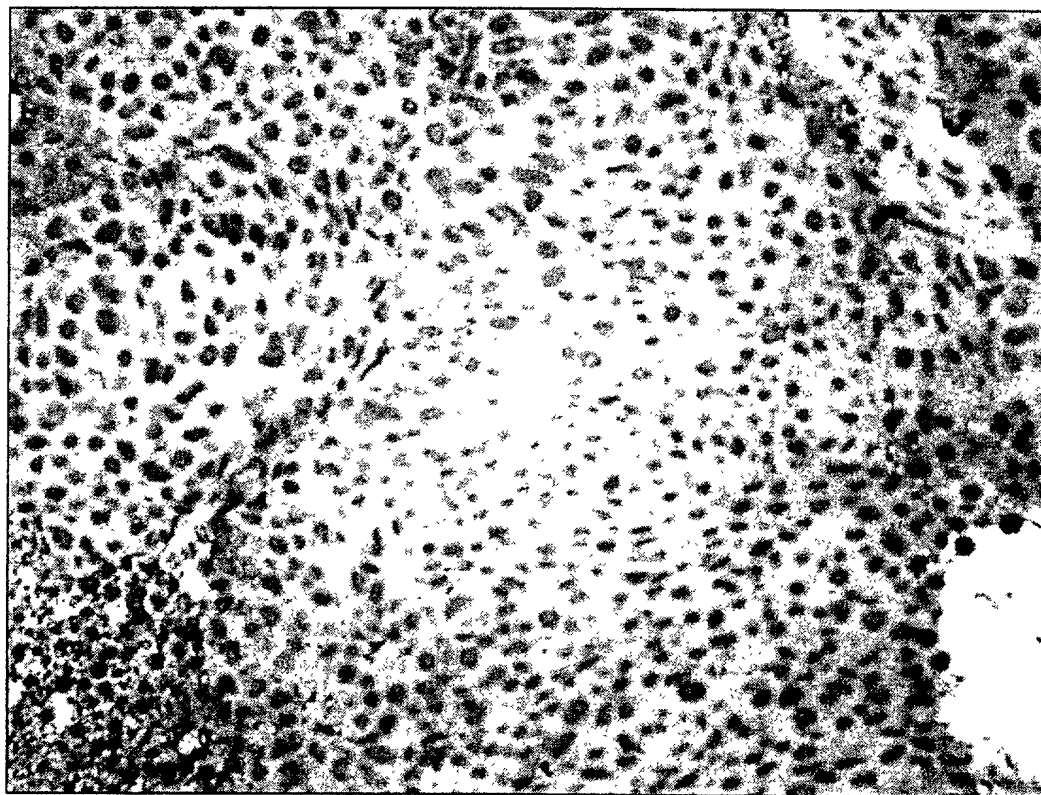


Figure 71

3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors

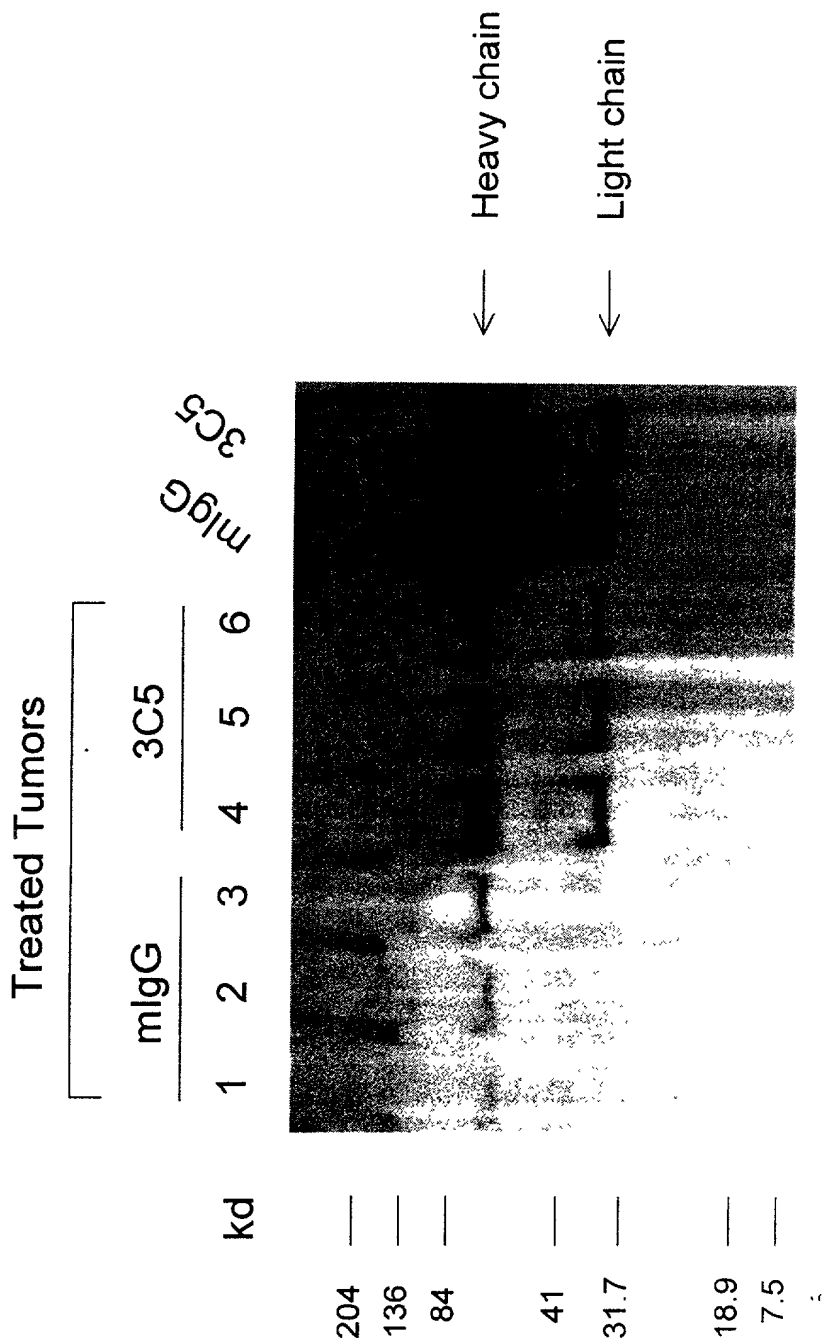
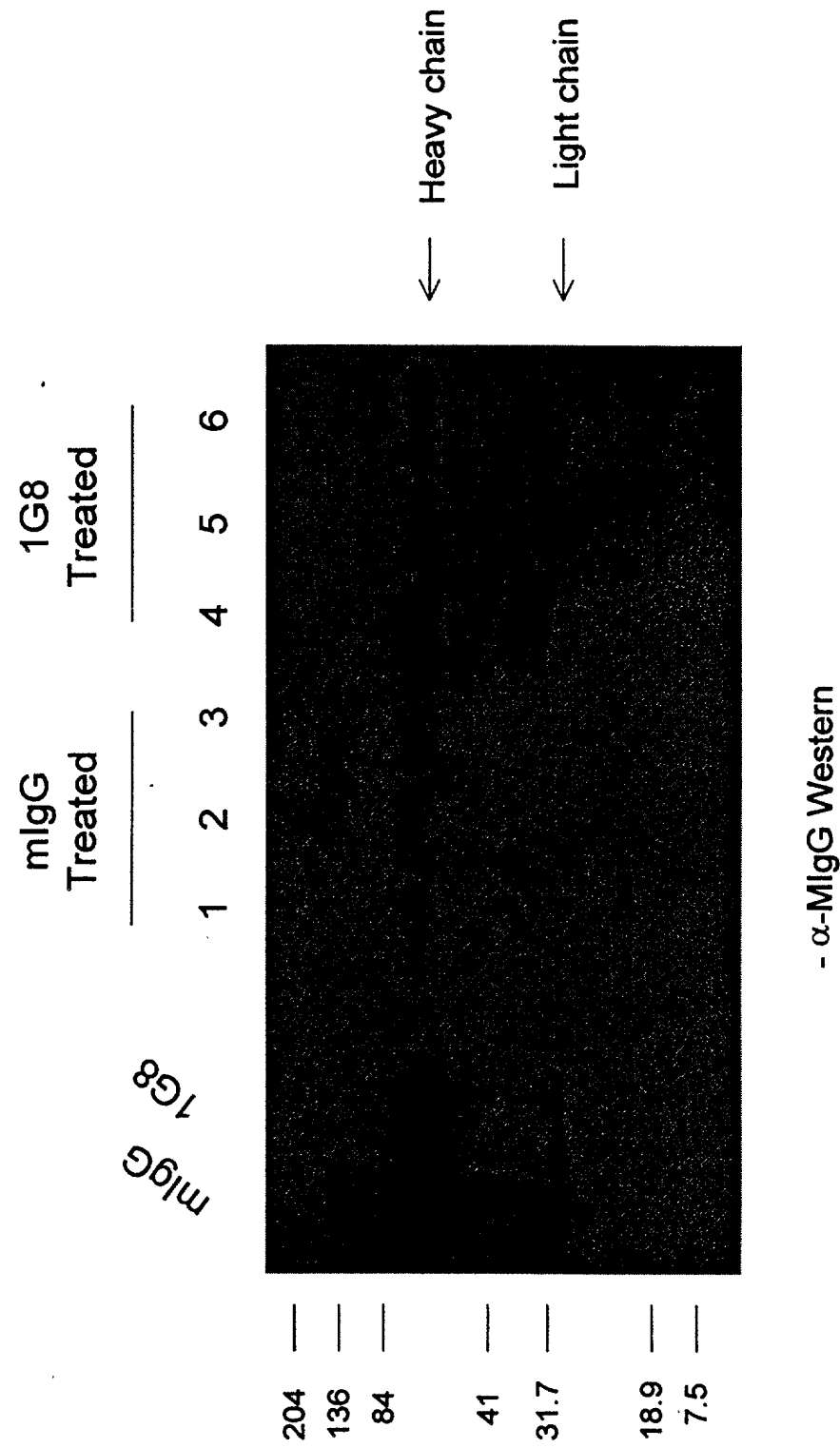


Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



Method: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

Figure 73